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# Analytical Protocols for Making a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media

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Toxic Substances
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# ANALYTICAL PROTOCOLS FOR MAKING A PRELIMINARY ASSESSMENT OF HALOGENATED ORGANIC COMPOUNDS IN MAN AND ENVIRONMENTAL MEDIA

by

Edo D. Pellizzari, Mitchell D. Erickson and R. A. Zweidinger

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# ABSTRACT

This comprehensive report presents the methods which will be used in Phase II of this program. Analytical methods for halogenated hydrocarbons in air, water, soil, breath, blood, urine, and tissue have been validated. A radioimmunoassay procedure for carcinoembryonic antigen (CEA) was validated. The data collection instruments (participant consent form, questionnaire, etc.) are also presented here.

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# LIST OF ABBREVIATIONS

CEA -- carcinoembryonic antigen assay
dpm -- disintegrations per minute
GC -- gas (liquid) chromatography
GC/ECD -- gas chromatography with electron capture detection or gas chromatograph with electron capture detector
GC/FID -- gas chromatography with flame ionization detection or gas chromatograph with flame ionization detector
GC/MS/COMP -- gas chromatography/mass spectrometry/computer or gas chromatograph/mass spectrometer/computer
LOD -- limit of detection
OMB -- Office of Management and Budget
PCB -- polychlorinated biphenyl
PCF -- Participant Consent Form
RIA -- radioimmunoassay
RMR -- relative molar response
SCOT -- support-coated open tubular (glass capillary GC column)
SIP -- single ion plot
SIM -- selected ion monitoring
WCOT -- wall-coated open tubular (glass capillary GC column)

# SECTION 1

# CONCLUSIONS

Methods have been assembled for (1) chemical analysis of halogenated organics in a variety of media, (2) carcinoembryonic antigen assay (CEA), and (3) data collection. The halogenated hydrocarbons of interest to this study include both volatiles (purgeable compounds such as dichloroethylene and dichlorobenzene) and semi-volatiles (extractable compounds such as tetrachlorobenzene and selected pesticides).

Methods have been validated for the sampling and analysis of purgeables and semi-volatiles in air, water, blood, urine, tissue, and soil. For most of the matrices, the compounds of interest are removed from the sample matrix, cleaned up, and analyzed by GC/MS/COMP.

Several clinical assays were considered but only CEA held any potential for yielding useful information. It is hoped that the CEA will provide an additional dimension to the correlation of the environmental exposure with health effects. CEA levels will be compared to body burden data to test the hypothesis that halogenated hydrocarbons elevate CEA.

The data collection instruments were developed, communication established with responsible authorities in the study areas, and a pretest completed.

This report will serve as a laboratory manual for those involved in subsequent phases of this project. The consolidation of all of the methods in a standard format will help assure the quality of the data. The use of these standard methods will also permit comparison of data collected in different areas and seasons.

## SECTION 2
## RECOMMENDATIONS

Future programs which undertake a comparative analysis between pollutants in the environment and in man should be extended to other chemicals which may be potentially responsible for the increase incidence of cancer in the Continental U.S. It is recommended however that a pilot program be instituted which provides the following information: (1) environmental levels of chemicals in air, water, soil, sediment and the food chain; (2) levels of the pollutants in the human biological fluids and tissue; (3) biological testing of chemicals for carcinogenic and mutagenic activity which is needed for the selection of the more important compounds for epidemiological studies; and (4) production and emission levels for chemicals during the past 10-15 years.

These data are required and are considered as the key ingredients in designing a program which makes an assessment of the organic pollutants in man and environmental media.

## SECTION 3

## PROGRAM OBJECTIVES

The general thrust of this program is to conduct environmental sampling and related epidemiological studies concerning halogenated organic compounds in five metropolitan locations within the Continental U.S. A comparative analysis is to be made of environmental levels (air, water, soil, sediment and aquatic and terrestrial species) with levels of chemicals found in the population and cancer mortality rates (in the metropolitan areas). The selected halogenated hydrocarbon chemicals are to be measured in breath, human blood, urine and/or fat tissues.

Under the first phase of this program, identification and quantification of the halogenated hydrocarbons occurring in air, water, etc. are to be performed for the sampling areas selected. The ultimate objective is to acquire information which will allow a statistical correlation of the levels of halogenated organic compounds in the environmental samples with those in human tissue and with the incidence of mortality.

The second phase is a comprehensive monitoring program of the five study areas over two seasons each. It is imperative that all sampling and analysis be conducted using standardized protocols so that the data may be properly correlated. This report presents these analytical protocols.

The third phase is an epidemiological study of the Phase II data.

# SECTION 4

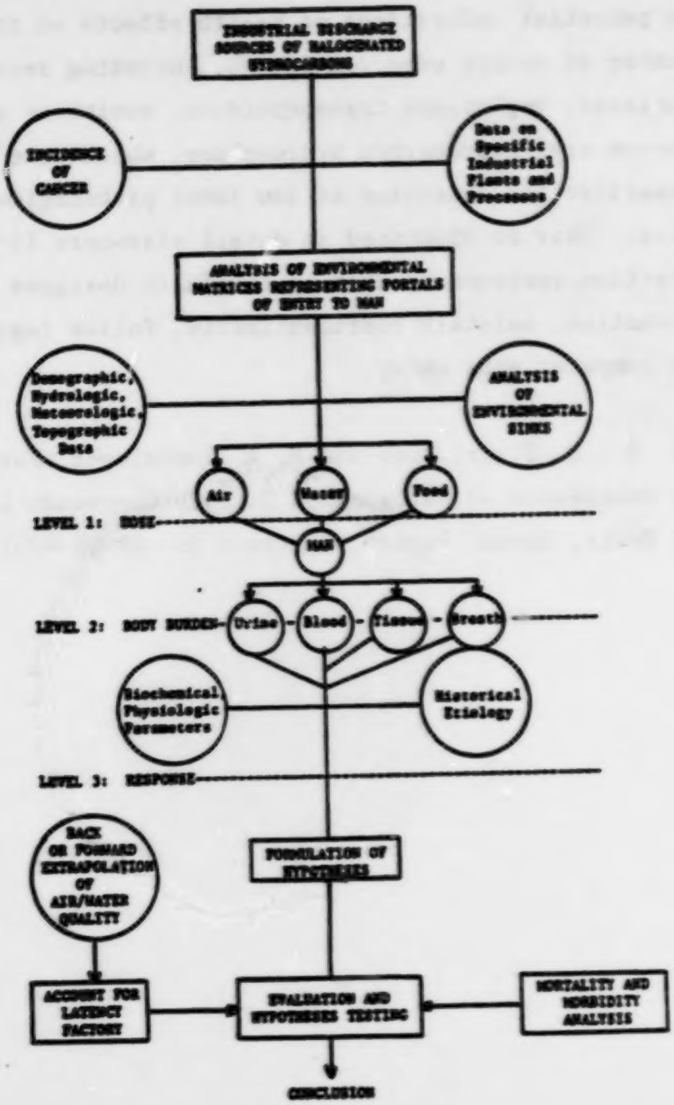
# INTRODUCTION

## GENERAL PROGRAM CONCEPT

The general program concept which has been developed attempts to furnish a comprehensive and systematic approach to making a comparative analysis of selected organic compounds in man and the environmental media. For the purpose of presenting an overview of the concept, Figure 4-1 depicts a flow diagram demonstrating the interlocking relationship between the environment, man and a potential association with the incidence of cancer. This schematic presents a number of prerequisite components of the current program which need examination to demonstrate a potential associational relationship between halogenated hydrocarbons and the incidence of cancer in man. The general program concept has been simplistically divided into three basic levels. The first demonstrates the dosage of man with halogenated hydrocarbons via environmental media such as air, water and food. The second demonstrates the degree of body-burden in man via examination of urine, blood, breath and tissue for these compounds. The last phase of the program attempts to demonstrate an associational relationship (i.e., response) between body-burden and the incidence of cancer.

## METHODOLOGY

In order that data collected from the various study areas and sampling times be comparable, it is imperative that all of the methodology be thoroughly standardized and validated. Some of the methods to be used are already in use at RTI and required only calibration or validation for the specific compounds of interest. Other methods have been either significantly altered from previous in-house procedures or were adapted from literature methods, and thus required thorough validation. It was the goal of the validation studies to define the precision and accuracy of each method and eliminate artifacts where possible.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ANALYSIS OF ENVIRONMENTAL SINKS
Air
Water
Food
LEVEL 1: DOSE
MAN
LEVEL 2: BODY BURDEN
Urine
Blood
Tissue
Breath
Biochemical Physiologic Parameters
Historical Etiology
LEVEL 3: RESPONSE
BACK OR FORWARD EXTRAPOLATION OF AIR/WATER QUALITY
FORMULATION OF HYPOTHESES
ACCOUNT FOR LATENCY FACTORS
EVALUATION AND HYPOTHESES TESTING
MORTALITY AND MORBIDITY ANALYSIS
CONCLUSION

**Figure 4-1.** Schematic flow diagram demonstrating interlocking relationships between environment, man and association with incidence of cancer.

In addition to the chemical sampling and analysis and the use of morbidity and mortality statistics, clinical blood chemistry and enzyme analyses were considered as potential indications of health effects on the study populations. A number of assays were considered, including serum glutamic-oxaloacetic transaminase, γ-glutamyl transpeptidase, ornithine carbamyl transferase, and serum aryl hydrocarbon hydroxylase, which were not sufficiently specific or sensitive for detection of low level pathological damage by halogenated organics. This is discussed in detail elsewhere (4-1).

The data collection instruments must be carefully designed to obtain all pertinent information, maintain confidentiality, follow legal guidelines and be amenable to computer data entry.

Reference

4-1 Pellizzari, E. D., M. D. Erickson and R. A. Zweidinger, Formulation of a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media, Annual Report, Contract No. 68-01-4731, July, 1979.

## SECTION 5

## COLLECTION AND ANALYSIS OF VAPOR-PHASE HALOGENATED ORGANICS

### INTRODUCTION

The methodology (Appendix A) for collection and analysis of vapor-phase halogenated organics has been previously described (5-1-5-8). These techniques have been successfully employed in Phase I of this program without any necessary modifications (5-8) and will be used in the final phase of this program.

### QUALITY CONTROL AND ASSURANCE

In addition to the procedures described in Appendix A, the following guidelines will be employed:

(1) 10% of all samples acquired will include "blank" sampling devices for determining background contributions,

(2) 10% of all samples will include "spiked" sampling cartridges for determining recovery of halogenated compounds selected for study

(3) Spiked samples will be generated from a permeation system, using gravimetrically calibrated permeation tubes

(4) During analysis every tenth sample will be a blank sampling cartridge followed by a spiked sample. The set of spiked samples will include external standards for calculating relative molar response ratio for quantification of unknowns.

(5) All samples will include a sampling protocol and chain of custody sheet indicating the history of the sample.

(6) Quality Assurance (QA) will include independent checks of collection and analysis procedures by an individual not involved in the procedure. QA by an independent laboratory may be included.

## REFERENCES

5-1 Pellizzari, E. D., Development of Method for Carcinogenic Vapor Analysis in Ambient Atmospheres", EPA-650/2-74-121, Contract No. 6802-1228, 148 pp., July, 1974.

5-2 Pellizzari, E. D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors, EPA-600/2-75-076, Contract No. 68-02-1228, 186 pp., November, 1975.

5-3 Pellizzari, E. D., The Measurement of Carcinogenic Vapors in Ambient Atmospheres, EPA-600/7-77-055, Contract No. 68-02-1228, 288 pp., June, 1977.

5-4 Pellizzari, E. D., Measurement of Carcinogenic Vapors in Ambient Atmospheres, EPA-600/7-78-062, Contract No. 68-02-1228, 224 pp., April, 1978.

5-5 Pellizzari, E. D., Improvement of Methodologies for the Collection and Analysis of Carcinogenic Vapors, EPA Contract No. 68-02-2764, *in press*.

5-6 Pellizzari, E. D., Analysis of Organic Air Pollutants by Gas Chromatography and Mass Spectroscopy", EPA-600/2-77-100, Contract No. 68-02-2262, 104 pp., June, 1977.

5-7 Pellizzari, E. D., Analysis of Organic Air Pollutants by Gas Chromatography and Mass Spectroscopy, EPA-600/2-79-057, Contract No. 68-02-2262, 243, pp., March, 1979.

5-8 Pellizzari, E. D., M. D. Erickson and R. A. Zweidinger, Formulation of a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media, EPA Contract No. 68-01-4731, Annual Report, *in prep*.

# SECTION 6

## COLLECTION AND ANALYSIS OF "PURGEABLE" HALOGENATED ORGANICS IN AQUEOUS ENVIRONMENTAL AND HUMAN SAMPLES

The purgeable halogenated organics are those compounds which are removed from liquid samples (water, urine, blood, etc.) by passing an inert gas stream through or over the surface of the sample and then collecting the evolved halogenated organics on a polymeric sorbent trap (e.g., Tenax GC). The technique, in varied forms, is routinely applied to water analysis. However, because of foaming and other problems, it has not been widely applied to biological matrices. Alternate techniques (e.g. solvent extraction) for extraction of volatile organics from blood and urine were not applicable due to a lack of sensitivity and the time required for analysis.

Under this contract, methods have been validated for analysis of purgeable halogenated organics in water, urine, blood, tissue, and soil. The methods are presented in Appendices B-E. Briefly, the sample is collected in a vapor-tight inert container (e.g. glass bottle with teflon-lined cap). The purgeable compounds are purged from an aliquot of the sample (either through solution or headspace purge) and trapped on a Tenax GC cartridge. The Tenax GC cartridge is then analyzed by GC/MS/COMP in a manner analogous to that for cartridges from air sampling. Appropriate quality control and quality assurance procedures are used. The purgeable halogenated organics range from gases up to the di- and trichlorobenzenes in volatility. It should be noted that the compound range for "purgeables" overlaps with that for "extractables" so there is no gap between the two procedures.

Two purging techniques, gas stripping and dynamic headspace analysis, have been validated. The former is used to purge water samples and the latter for the biological samples.

The approach to the validation of these techniques involved complementary, parallel studies: 1) recovery studies with radiolabeled compounds and 2) recovery studies with non-radiolabeled substances. The experiments with radiolabeled compounds addressed recovery on the basis of the percentage of fortified material detected on the sorbent cartridge and also on the basis of how much remained in the sample following analysis. This experimental approach permitted construction of a "mass balance" for the analysis. Experiments with non-radiolabeled compounds involved compounds comprising a range of volatility to simulate the procedure to be employed in the analysis of actual samples. The use of two independent validation procedures assured not only the precision and accuracy of the analytical results but also guarded against systematic errors.

The sections below discuss the validation of the methods to be used for sampling and analysis of "purgeable" halogenated organics in detail.

## BACKGROUND OF PURGE AND TRAP PROCEDURE

A number of techniques developed for the determination of volatile organic compounds in water, particularly drinking water, were considered for application to water and human tissue samples. The methods fall into six basic categories: 1) solution purge (gas stripping) (6-1,6-2,6-3,6-4); 2) dynamic headspace purge (6-5,6-6); 3) solvent extraction (6-7); 4) liquid phase adsorption on polymeric sorbents (6-8,6-9,6-10); 5) direct aqueous injection (6-11,6-12) and 6) static headspace analysis (6-13). Solvent extraction, using high- or low-boiling solvents, generally suffers from poor sensitivity due to the large volume of solvent relative to the amount injected. Direct aqueous injection lacks adequate sensitivity for these analyses due to injection volume limitations and in addition would present large matrix interferences. Adsorption of volatile organics from a tissue matrix directly onto polymeric sorbents such as XAD or polyurethane foam is also highly susceptible to matrix interferences. Static headspace analysis requires knowledge of the equilibrium coefficients and rigid control of the sample temperature, headspace volume, and other parameters. In addition, except for compounds which strongly favor the gas phase (e.g. highly volatile, water-insoluble substances), static headspace analysis lacks sufficient sensitivity. In light of these difficulties, actual experimental investigations were performed only for gas stripping and dynamic headspace techniques in combination with vapor collection on Tenax GC.

Gas stripping analysis involves bubbling an inert gas through the solution to purge the volatile organic materials from the sample, followed by collection of the vapor on a polymeric sorbent. This technique has been shown to be extremely sensitive for water-insoluble organics with boiling points less than approximately 150°C (6-1). Dynamic headspace analysis is similar to gas stripping in its dependence on the partition coefficient of the solute between the aqueous and gas phases except the inert gas passes over, rather than through the solution.

Tenax GC was chosen as the sorbent for these experiments based on previous successful use in purge and trap analyses of water (6-1) its low affinity for water (6-14), its low background compared with other sorbents (6-15) and our previous work to determine breakthrough volumes and thermal desorption conditions (6-16).

## BACKGROUND OF GC/MS ANALYTICAL PROCEDURE

Gas Chromatography/Mass Spectrometry/Computer (GC/MS/COMP) has been selected as the analytical technique for this program. This selection was based on previous knowledge of the complexity of the purgeable organic fraction of water and biological samples. Other methods of chromatographic detection (electron capture, electrolytic conductivity, etc.) do not provide sufficient identification to verify the presence of the halogenated organics of interest.

In addition to the need for mass spectrometric detection, it is necessary to use high resolution gas chromatography to separate the complex mixtures which are purged from the water and biological samples. High resolution GC is achieved by the use of capillary columns.

In order to limit the scope of the program and concentrate on the compounds of highest concentration and highest carcinogenic potential, the number of compounds of interest has been restricted. One problem with this approach is the potential exclusion of a compound which may later be of interest. Therefore, where possible, full spectral data are collected and archived on magnetic tape. If this data is subsequently needed it may be retrieved.

Even though full scan spectra are collected, their printout and interpretation are time-consuming. A more efficient technique, mass fragmentography, consists of printing out on paper or scope display the chromatogram of an ion(s) selective for certain compounds. If the retention time matches that of the standard, the peak may be quantitated using the Relative Molar Response (RMR). Identifications may be verified by comparing intensities of fragmentograms of different ions for the same chromatographic peak to see if the intensity ratio matches that of a standard. Details of the approach may be found in Appendices B-E.

## GENERAL METHOD VALIDATION PROCEDURES

The validation of the methods for analysis of purgeable halogenated organics in various matrices required similar procedures. Rather than repeat the general experimental protocols for each medium, they are discussed in the sections below.

### Materials and Methods

All chemical compounds in this study were reagent grade (various sources) and were used without further purification. Carbon-14 labeled chloroform (California Bionuclear Corp.), carbon tetrachloride (Amersham Corp.), chlorobenzene (Amersham Corp.) and bromobenzene (New England Nuclear) were quantitatively transferred to 50 ml of redistilled methanol in screw cap vials. Solvents were exclusively "distilled-in-glass" grade (Burdick and Jackson Laboratories). Working solutions were prepared at the concentrations listed in Table 6-1. Safety precautions were observed while handling radioisotopes, solutions thereof, Tenax GC cartridges containing isotopically-substituted compounds, and contaminated glassware.

Tenax GC was purified before use by Soxhlet extraction for 24 hours each in redistilled methanol and pentane. After vacuum drying at 60°C for 18 hr and sizing into 35/60 mesh, 1.5 cm i.d. x 6.0 cm cartridges were packed and thermally desorbed at 270° in a stream of helium for 30 min.

Gas chromatographic analyses were performed on either a Varian 3700 or a Varian 1400 gas chromatograph with flame-ionization detection (GC/FID). Exposed sample cartridges and standard cartridges were analyzed by GC/FID on a 0.35 mm i.d. x 80 m, 1% SE-30 glass SCOT column which was temperature programmed from 30 to 220°C at 4°/min. Compounds were removed from the

## Table 6-1. [$^{14}$C] COMPOUNDS FOR RECOVERY STUDY

| Compound[a] | Source | Radiochemical Purity | Specific Activity | MW (g/mol) | bp, °C | Methanol Solution Concentration (dpm/ml) |
|---|---|---|---|---|---|---|
| [$^{14}$C]-$CHCl_3$ | California Bionuclear Corp. | 98% | 3.4 mCi/mmol | 119.38 | 62 | 44,400 |
| [$^{14}$C]-$CCl_4$ | Amersham | 97% | 6.94 mCi/mmol | 157.82 | 76 | 22,200 |
| [$^{14}$C]-chlorobenzene | California Bionuclear Corp. | 98% | 4.1 mCi/mmol | 112.56 | 132 | 44,400 |
| [$^{14}$C]-bromobenzene | New England Nuclear | not specified | 5 mCi/mmol | 157.02 | 156 | 55,500 |

[a] All compounds universally labeled with $^{14}$C.

[b] dpm = disintegrations per minute.

Tenax GC cartridge for injection into the GC by thermal desorption at 250°C for 5 min in a nitrogen stream (30 ml/min) and trapped in a liquid nitrogen-cooled, nickel capillary. Upon heating this trap to 250°C, the desorbed compounds were injected onto the column in a minimum volume. This procedure has been previously described in detail (6-17). Scintillation counting was achieved on a Packard-TriCarb Model 3255 liquid scintillation counter. Counting cocktail was prepared by mixing toluene (3.0 l), Triton X (1.0 l) and Omnifluor (15 g, New England Nuclear).

The purgeable halogenated organics were analyzed by GC/MS/COMP using an LKB 2091 GC/MS with an LKB 2031 data system equipped with a desorption chamber(6-17). The operating conditions for the thermal desorption unit and the GC/MS system for the analysis of Tenax GC cartridges were as follows:

Desorption chamber temperature = 270°C
Desportion chamber He flow = 20 ml/min
Desorption time = 5.0 min
Capillary trap temperature during desorption = -196°C
Temperature of capillary trap during injection onto
    column = -196°C to 250°C then held at 190°C
Time of He flow through capillary trap = 12 3/4 min
He flow through column (sweep time) = 9.5 min after injection
Capillary column = 100m SE-30 Column temperature = 30°/2 min;
    increased at 4°/min; to 240°
Scan range = 5 → 490 daltons
Scan cycle time = 2.4 sec
Scan mode = parabolic
Trap current = 50 µA
Ionizing energy = 70 eV
Accelerating voltage = 3.5 kV

## Purge and Trap Apparatus

Three apparatuses, shown in Figures 6-1, 6-2, and 6-3 have been used. The gas stripping apparatus for water samples (Figure 6-1) combines a uniform dispersion of fine helium bubbles throughout the solution with a minimum of headspace. This apparatus has proven satisfactory in repeated applications.

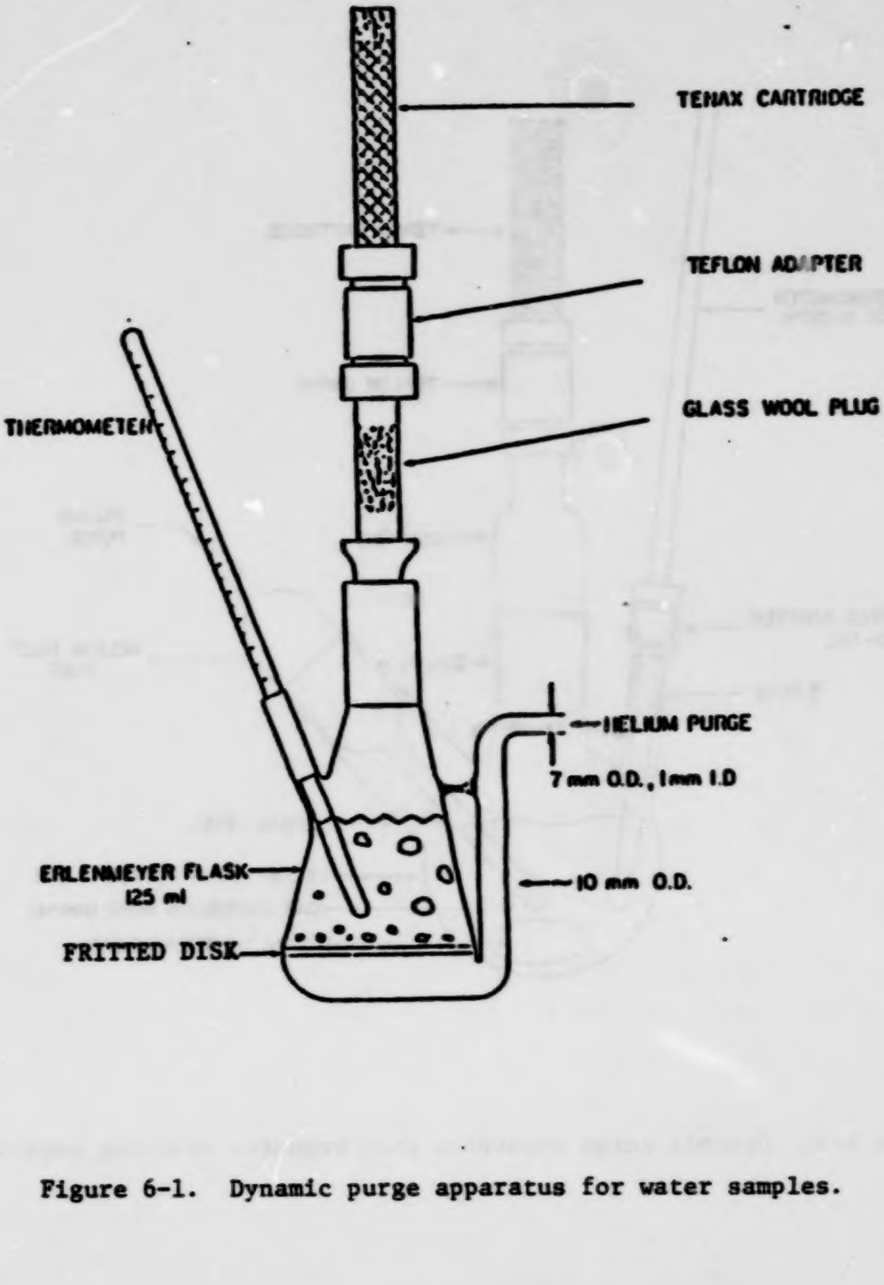
TENAX CARTRIDGE
TEFLON ADAPTER
GLASS WOOL PLUG
THERMOMETER
HELIUM PURGE
7 mm O.D., 1 mm I.D
ERLENMEYER FLASK
125 ml
10 mm O.D.
FRITTED DISK

Figure 6-1. Dynamic purge apparatus for water samples.

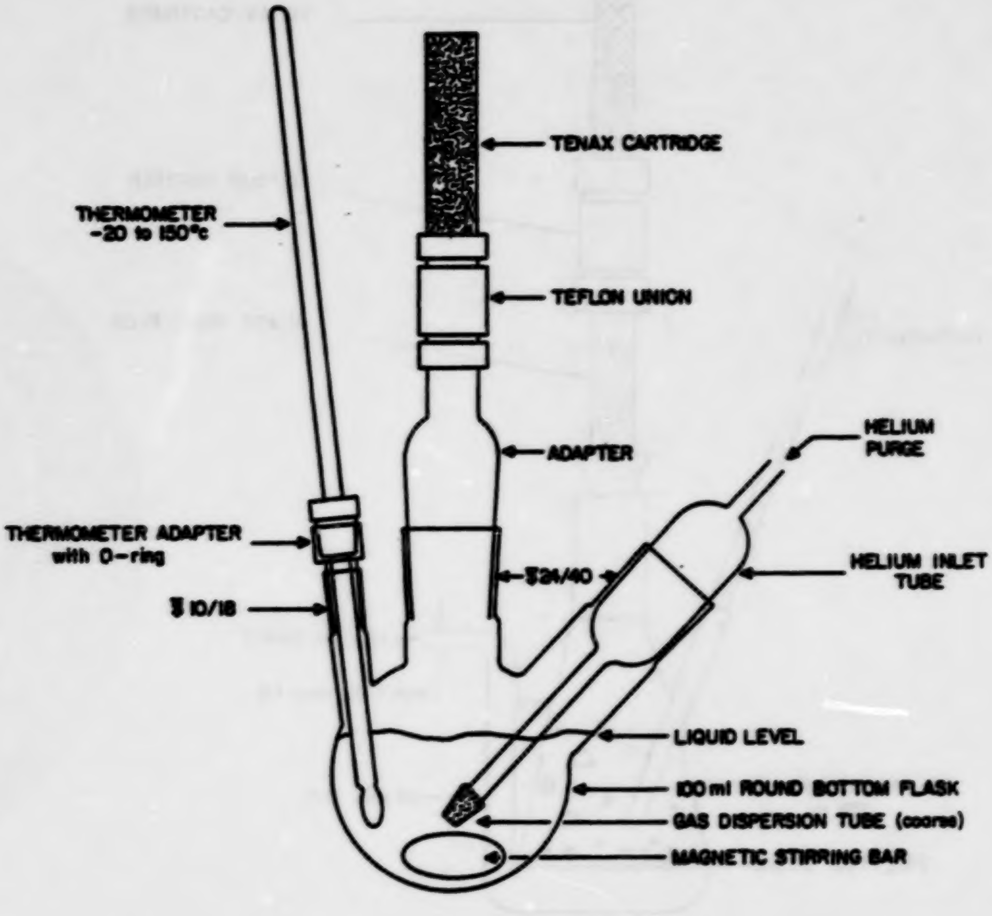
TENAX CARTRIDGE
THERMOMETER -20 to 150°c
TEFLON UNION
ADAPTER
HELIUM PURGE
THERMOMETER ADAPTER with O-ring
HELIUM INLET TUBE
₸ 24/40
₸ 10/18
LIQUID LEVEL
100 ml ROUND BOTTOM FLASK
GAS DISPERSION TUBE (coarse)
MAGNETIC STIRRING BAR

Figure 6-2. Dynamic purge apparatus with magnetic stirring capability.

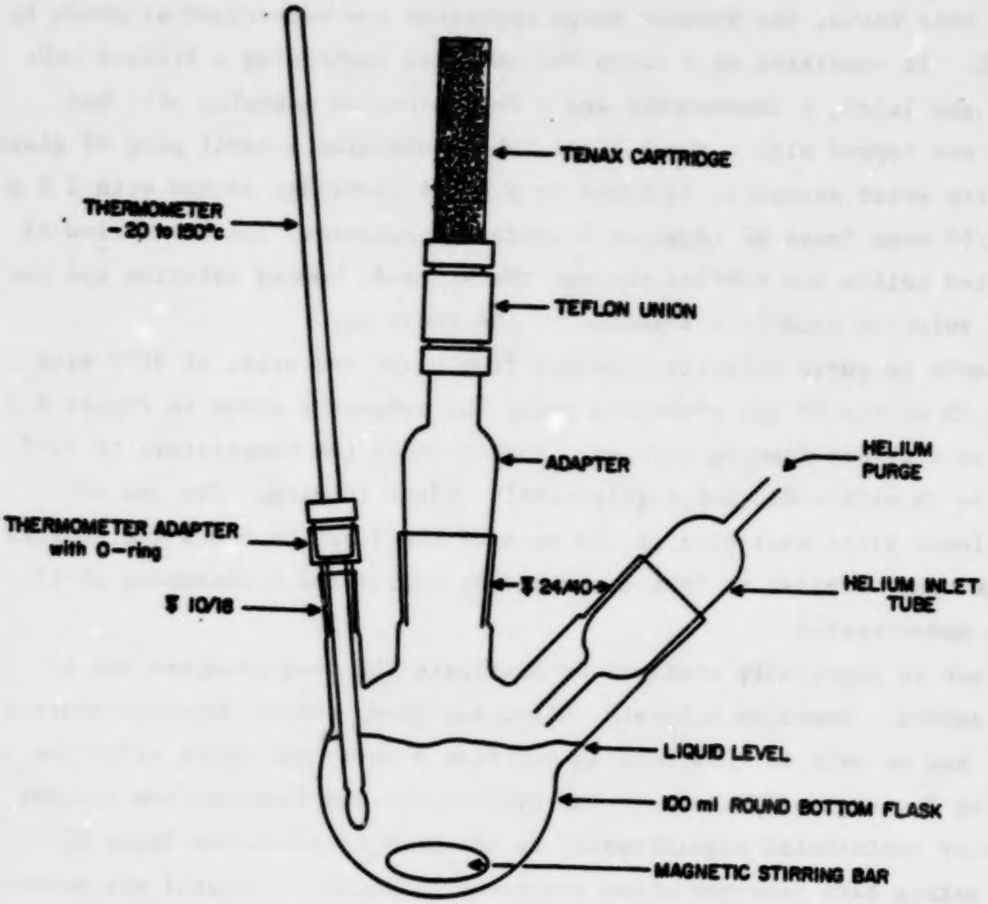
TENAX CARTRIDGE
THERMOMETER
-20 to 150°c
TEFLON UNION
ADAPTER
HELIUM PURGE
THERMOMETER ADAPTER with O-ring
₸ 24/40
₸ 10/18
HELIUM INLET TUBE
LIQUID LEVEL
100 ml ROUND BOTTOM FLASK
MAGNETIC STIRRING BAR

**Figure 6-3.** Headspace purge apparatus for blood, urine, and tissue samples.

The biological samples tend to precipitate upon heating and must be stirred to adequately mix during purging. This precluded the use of the apparatus in Figure 6-1, since a stirring bar would wear through the glass frit. On this basis, the dynamic purge apparatus was redesigned as shown in Figure 6-2. It consisted of a round-bottom flask containing a fritted tube for purge gas inlet, a thermometer and a Teflon-coated magnetic stir bar. The flask was topped with a short glass tube containing a small plug of glass wool to trap water aerosols, followed by a glass cartridge packed with 1.5 x 6.0 cm 35/60 mesh Tenax GC (Applied Science Laboratories, Inc.). A flow of pre-purified helium was bubbled through the stirred, heated solution and the entrained volatile materials adsorbed by the Tenax GC.

Attempts to purge volatile organics from blood and urine at 90°C with helium at 25 ml/min by gas stripping using the apparatus shown in Figure 6.2 resulted in a severe foaming problem. Reduction of the temperature to 30°C and flow to 15 ml/min did not significantly reduce foaming. The use of either a loose glass wool plug in the neck of the flask to break the bubbles or a "foam trap" similar to that described by Bellar and Lichtenberg (6-1), were also unsuccessful.

Failure to physically obstruct or dissipate the foam prompted use of chemical agents. Ammonium sulphate, which has been used to denature protein material, had no effect. Dow Corning Antifoam A spray was quite effective in controlling foaming; unfortunately the hydrocarbon and fluorocarbon content of the spray contributed significantly to the background on the Tenax GC sorbent, making data interpretation extremely difficult. Octanol was moderately successful as an antifoaming agent but was too volatile for this application.

Dynamic headspace purge analysis of volatile compounds in biological media, like gas stripping analysis, depends on the partition between the aqueous and gas phases and the surface area of the interface. The partition is affected by a number of factors including temperature and solubility of the compounds of interest in water. Although the gas-water interfacial area is significantly smaller in the headspace technique than in gas stripping and hence the rate of transport of volatile materials from the liquid to gas phase is diminished, several parameters can be adjusted to optimize the purge efficiency in headspace analysis. Higher temperatures are possible since the gas does not flow through the solution and foaming is not a concern. Furthermore, vortexing the solution with rapid stirring increases the interfacial area.

In order to prevent foaming, the apparatus shown in Figure 6-2 was modified as shown in Figure 6-3. This apparatus for dynamic headspace purging performed satisfactorily with blood, urine, tissue, and other biological samples during preliminary tests. Accordingly, it was used in the validation studies discussed below.

## Recovery Studies With Radiolabeled Compounds

Recovery studies using carbon-14-labeled chloroform, carbon tetrachloride, chlorobenzene and bromobenzene were performed to assess the percentages of material collected by the Tenax GC trap, loss through connections in the apparatus and remaining in the sample matrix. This allowed construction of a mass balance for the analysis. Recoveries were assessed for the sorption/desorption process alone, the gas stripping technique, and the dynamic headspace technique.

Residue samples were oxidized using the Packard Tri-Carb Combustion Analyzer. A 1 ml aliquot was completely oxidized to $H_2O$ and $CO_2$; the $CO_2$ was trapped in an absorbing solution and a scintillation "cocktail" (Permafluor® in 1.0 l toluene) in a second series of scintillation vials.

To isolate the purging efficiency from the sorption/desorption process, recoveries of compounds loaded directly onto Tenax GC cartridges were evaluated. The apparatus illustrated in Figure 6-4 was used to load the model compounds onto the cartridge. Methanol solutions of the carbon-14 labeled compounds were injected through the septum of the loading tube into a heated (106°C) chamber where they were vaporized and swept into the Tenax cartridge in a $N_2$ stream of 20 ml/min. Approximately 15 minutes were required to vaporize and load up to 67.6 µl (0.75- 1.42 µg). Loaded cartridges were assayed by thermal desorption for 10 minutes in a desorption chamber (6-17) at 270°C under a He flow of 30 ml/min. Effluent gas containing the desorbed compounds was bubbled into two vials in tandem containing 15 ml of scintillation counting cocktail. These samples were subsequently analyzed by liquid scintillation counting. Triplicate standards of each compound were prepared by injecting known amounts

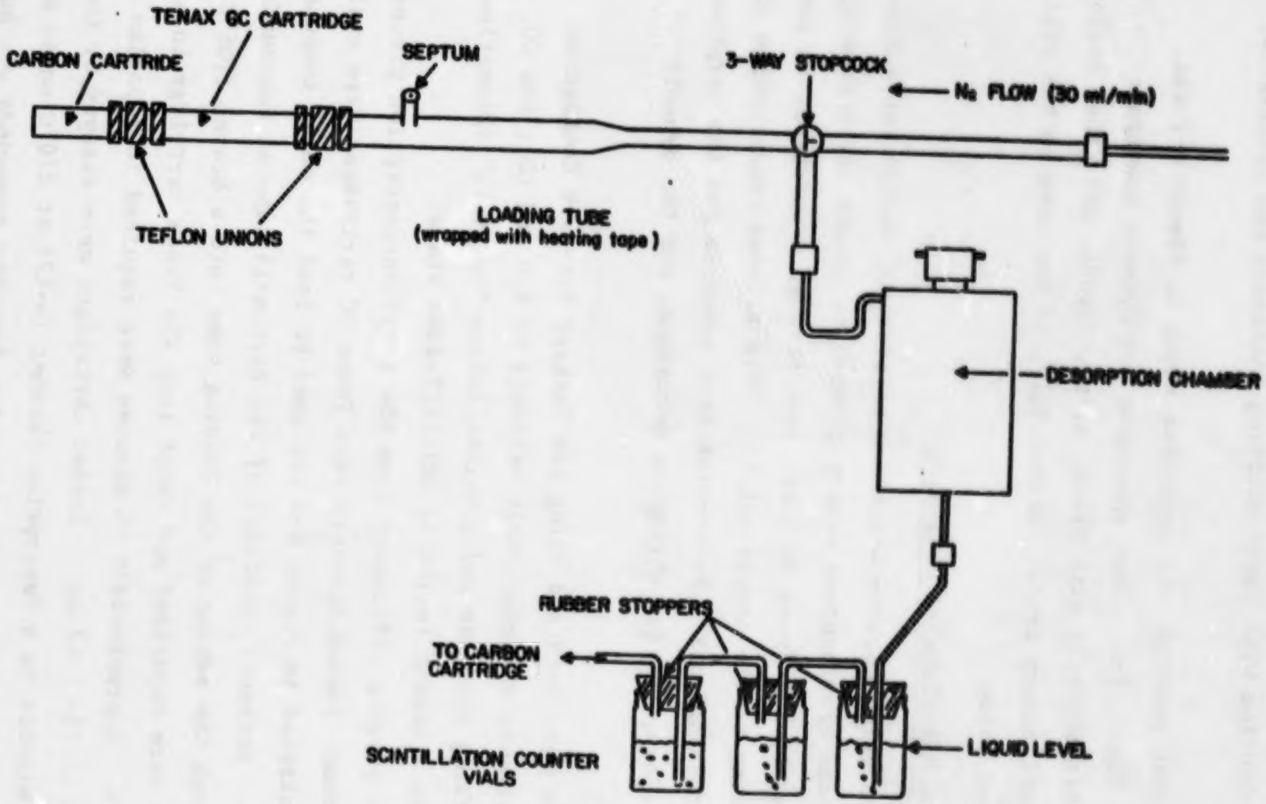
CARBON CARTRIDE
TENAX GC CARTRIDGE
SEPTUM
3-WAY STOPCOCK
$N_2$ FLOW (30 ml/min)
TEFLON UNIONS
LOADING TUBE
(wrapped with heating tape)
DESORPTION CHAMBER
RUBBER STOPPERS
TO CARBON CARTRIDGE
SCINTILLATION COUNTER VIALS
LIQUID LEVEL

Figure 6-4. Tenax cartridge loading and desorption apparatus for use with radiolabeled volatile compound.

of radiolabeled material directly into counting vials containing 15 ml of cocktail. Carbon cartridges backed up both the Tenax GC cartridge during loading and the scintillation vials during desorption and trapping to retain any radioactive material which escaped. Appropriate safety precautions were observed in the handling of radioisotopes.

Thermal desorption of radiolabeled compounds from Tenax GC cartridges directly into scintillation cocktail was found to be very efficient as shown in Table 6-2. The low recovery of carbon tetrachloride was attributed to incomplete loading of this relatively large amount of material rather than incomplete desorption. Less than 1% of the total amount of each compound was found in the second impinger, indicating that the compounds were not breaking through the scintillation cocktail. A second desorption of a loaded Tenax cartridge, identical to the first desorption after loading, extracted an average of 0.06% of the total dpm loaded on the cartridge. To investigate the possibility of breakthrough of compounds from the cartridge during loading, a second cartridge was placed behind several of the cartridges and was desorbed into Triton X cocktail, which was scintillation counted. The second cartridge contained from 0.9 to 3.0% of the total dpm loaded.

When large volumes of radioisotope solutions (160-689 µl) were injected into the loading tube, recoveries decreased in direct proportion to the total volumes of solution injected. Apparently this quantity of methanol competed with chloroform and carbon tetrachloride for absorption sites on the Tenax GC, causing the labeled compounds to pass through the sorbent bed without being adsorbed.

In summary, thermal desorption of the labeled compounds from Tenax GC was sufficiently reproducible and efficient to be a technique useful for completing recovery studies of the purging of volatile compounds from various sample media.

Combusted samples, Tenax eluent samples, blanks and standards were counted for 5 min periods on a Packard scintillation counter. Data was recorded via teletype and paper-tape punch. Computer programs at TUCC (Triangle Universities Computation Center) were used to reduce the data.

Table 6-2. RECOVERY OF $^{14}$C-LABELED COMPOUNDS FROM TENAX GC BY THERMAL DESORPTION

| Compound Loaded | Volume Loaded (μl) | Average Loaded (μl) | Average dpm[a] | % Recovery[b] (± Std. dev.) |
|---|---|---|---|---|
| chloroform | 16.1 | 0.56 | 87436.5 | 97.1 ± 1.91 |
| carbon tetrachloride[c] | 67.6 | 0.75 | 73764.2 | 89.6 ± 8.25 |
| chlorobenzene | 29.7 | 1.24 | 85076.9 | 98.9 ± 0.59 |
| bromobenzene | 12.6 | 1.42 | 80074.8 | 96.5 ± 2.08 |

[a] Based on three cartridges.

[b] First cartridge, first impinger only.

[c] Nine replicates.

## Recovery Studies with Non-Radiolabeled Compounds

The recovery efficiencies of model compounds were determined in a manner analogous to that used with radiolabeled compounds except for the sample fortification technique and the analysis of compounds desorbed from the Tenax GC cartridge.

Standard mixtures of methylene chloride, chloroform, bromodichloromethane, tetrachloroethylene, chlorobenzene and m-dichlorobenzene were prepared in dry, pre-purified nitrogen gas at approximately 75°C by injecting small amounts (1-5 µl) of the pure liquid into a 2.0 l spherical glass bulb. Mixtures were equilibrated with stirring for one hour. Using a gas tight syringe (Precision Sampling) a 1.0 ml aliquot of the vapor standard was injected into the sample contained in a septum-capped bottle at 4°C. Other means of loading standards were investigated, as discussed in the following section. Quantitation standards were prepared by loading 1.0 ml aliquots of the vapor standard directly into Tenax GC cartridges. After allowing the spiked water samples to eqilibrate at 4°C for one hour, they were transferred to the apparatus shown in Figure 6-1. The solution itself was purged for 90 minutes with 25 ml He/min. Exposed Tenax GC cartridges were desiccated for 12 hours by storing in capped culture tubes containing approximately 2 g of a calcium sulfate. This effectively removed the large amount of adsorbed water vapor from the cartridge which would have interfered with thermal desorption of the cartridges and would have degraded gas chromatographic columns. The cartridges were analyzed by thermal desorption/GC/FID.

## Investigation of Methods for Loading Standards

Several procedures for loading standards to the aqueous samples were attempted. The vaporization of liquid samples in a glass bulb described above does not take into account wall adsorption, incomplete volatilization, and syringe leaks which may reduce the measured recovery. The other procedures tried, spiking with solutions in methanol and toluene and the use of gas permeation tubes were unsuccessful as discussed below.

The use of methanol solutions for spiking water samples is routine (6-18). This procedure was used to spike distilled water samples. The results of the analyses of four samples spiked with methanol solution are presented in Table 6-3. The results indicate a wide range of recoveries

Table 6-3. RECOVERY OF HALOGENATED HYDROCARBONS FROM DISTILLED WATER SPIKED IN METHANOL AND TOLUENE SOLUTIONS

| Compound[a] | Amount Spiked (μg) | Boiling Point (°C) | % Recovery |  |  |  | Average |
|---|---|---|---|---|---|---|---|
|  |  |  | 1 | 2 | 3 | 4 |  |
| methylene chloride |  | 40 | b |  |  |  |  |
| chloroform |  | 61.7 | b |  |  |  |  |
| 2-chloro-1,3-butadiene |  | 59.4 | b |  |  |  |  |
| 1,1,1-trichloroethane |  | 74.1 | b |  |  |  |  |
| 1,2-dichloroethane |  | 83.5 | b |  |  |  |  |
| bromodichloromethane[c] | 2.50 | 90 | 12.8 | 14.8 | 36.4 | 36.4 | 25.1 |
| 1,2-dichloropropane[c] |  | 96.4 |  |  |  |  |  |
| tetrachloroethylene | 1.30 | 121 | 139 | 161 | 211 | 217 | 182 |
| chlorobenzene | 0.884 | 132 | 67.9 | 76.9 | 95.0 | 95.0 | 83.7 |
| m-dichlorobenzene | 1.03 | 173 | 52.4 | 56.3 | 96.1 | 87.4 | 73.0 |

[a] The first 5 spiked in toluene solution; the last 5 spiked in methanol solution.

[b] Analysis of Tenax cartridges unsuccessful because of trap freezing with toluene.

[c] Not separated by glc.

from about 25% to 182%. Due to the imprecision found and the interference of the methanol peak in the gas chromatographic analysis, this technique was abandoned.

Analysis of Tenax cartridges loaded by purging distilled water spiked with standards in toluene was unsuccessful as shown in Table 6-3. The high breakthrough volume of toluene on Tenax resulted in quantitative adsorption of the 10 µl used to spike the standards. Thermal desorption of these toluene-laden cartridges immediately prior to GC/FID analysis plugged the cryogenic capillary trap with the large volume of solidified toluene. Consequently the transfer of desorbed materials from the Tenax to the trap ceased. Methanol, having a very low breakthrough volume on Tenax, did not pose this problem. However, it could not be used as a solvent for the more volatile compounds.

Another procedure for fortifying aqueous samples with halogenated hydrocarbons was attempted using gas permeation tubes. A stream of purified $N_2$ passing over a group of permeation tubes in a thermostated chamber entrains a low concentration of vapor as shown in Figure 6-5. Permeation rates, determined gravimetrically by weighing each tube at periodic intervals, ranged from $1 \times 10^{-6}$ to $\sim 1 \times 10^{-10}$ g/min/cm using tubes constructed of TFE, FEP and polyethylene.

Fortification of distilled water by placing the purge flask (Figure 6-1) with 100 ml water in-line in the gas permeation system (Figure 6-5) appeared, on the surface, to be the most straight forward and accurate procedure. Unfortunately the backpressure created by the sintered-glass frit and the 100 ml of water was sufficient to stop the gas flow in the permeation system. Leakage of gas from the many fittings in the system was not correctable and the procedure was not investigated further.

An alternate fortification procedure involved withdrawing aliquots of gas from a septum port of the permeation system with a gas-tight syringe. These gas aliquots were then injected into 60 ml of distilled water samples contained in septum-capped, amber-glass bottles. Tenax cartridges were loaded similarly and were used as standards. After equilibration for approximately 1 hr at 4°C, the solution was quickly transferred to a purge flask topped with a Tenax cartridge and diluted to 100 ml with distilled water.

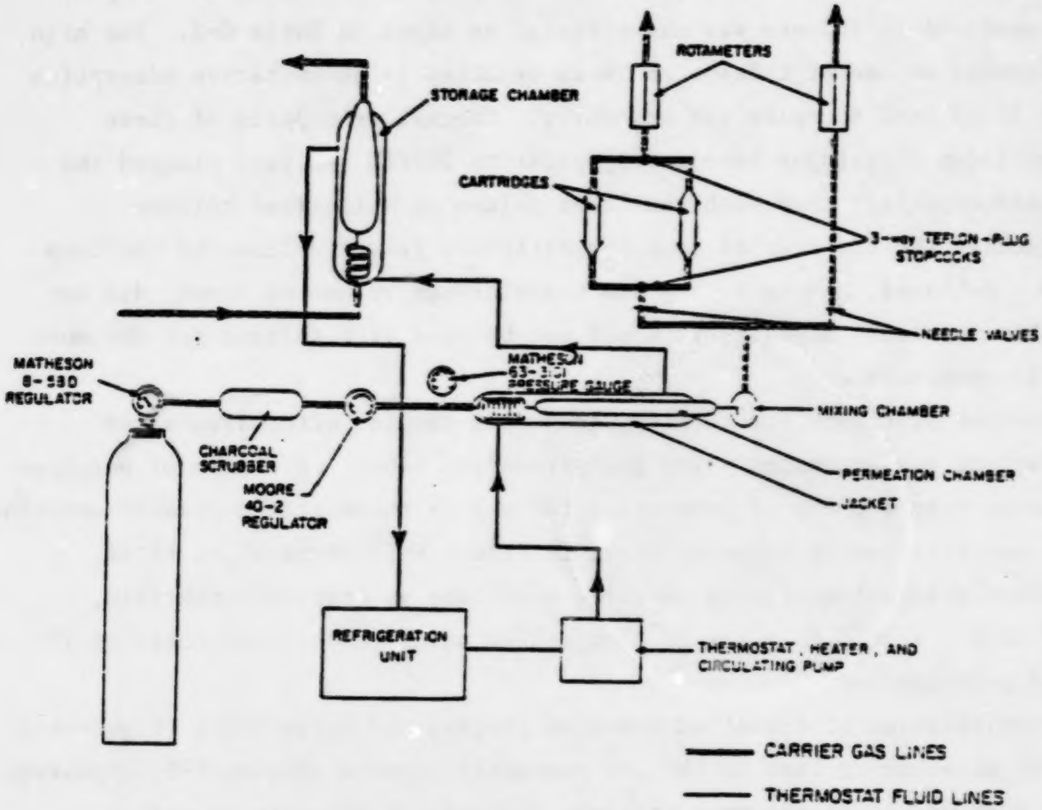
ROTAMETERS
STORAGE CHAMBER
CARTRIDGES
3-way TEFLON-PLUG STOPCOCKS
NEEDLE VALVES
MATHESON 8-580 REGULATOR
MATHESON 63-3101 PRESSURE GAUGE
MIXING CHAMBER
CHARCOAL SCRUBBER
MOORE 40-2 REGULATOR
PERMEATION CHAMBER
JACKET
REFRIGERATION UNIT
THERMOSTAT, HEATER, AND CIRCULATING PUMP
CARRIER GAS LINES
THERMOSTAT FLUID LINES

Figure 6-5. Schematic of permeation tube flow system for synthesizing air/vapor mixtures.

The solution was heated to 90°C and purged for 90 min at 25 ml He/min. Vapors collected on the Tenax were detected and quantitated by GC/FID interfaced with a thermal desorption/injection system. The results appear in Table 6-4.

The fourth method of fortification involved preparation of vapor standards in a gas mixing bulb. Two microliters of each compound were injected into the bulb at 75°C and the vapor mixture stirred for 30 min. Aliquots (1 ml) were withdrawn with a gas-tight syringe and injected into 60 ml of distilled water contained in a septum-capped, amber glass bottle. The solutions were analyzed according to the procedure in the previous paragraph. The results are presented in Table 6-5.

Comparison of the results of the last two spiking procedures reveals similar recovery. However, the precision shown when spikes were made from the gas mixing bulb is approximately a factor of 2 better than that shown when spikes were made by withdrawing aliquots from the permeation system. Consequently fortification of samples for recovery studies was accomplished from the gas mixing bulb.

## VALIDATION OF GAS CHROMATOGRAPHY/MASS SPECTROMETRY/COMPUTER ANALYSIS PROCEDURES

The GC/MS/COMP procedures to be used for the purgeable halogenated organics are routine in this laboratory and do not require extensive validation. One area which requires ongoing investigation is the determination of Relative Molar Response (RMR) factors for use in quantification. Details on the use and determination of RMR may be found in the Appendices. Due to instrumental variations, RMRs may change slightly with time. Therefore, RMR values are periodically checked and updated if necessary.

As part of this program, RMRs were obtained for many of the purgeable halogenated organics of interest. Using the LKB 2091 GC/MS/COMP system, a series of Tenax GC cartridges were loaded with standards for the determination of Relative Molar Response factors in order to calibrate this instrument for subsequent quantification of the purgeable halogenated organics in water, urine and blood samples. The RMRs for standard compounds (determined in replicate) were calculated relative to the m/z 186 ion of perfluorobenzene. These data are given in Table 6-6.

Table 6-4. RECOVERY OF HALOGENATED HYDROCARBONS FROM DISTILLED WATER SPIKED FROM GAS PERMEATION SYSTEM

| Compound | Amount spiked (μg) | % Recovery | | | | | | Average ± Std. Dev. |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| *trans*-1,2-dichloroethylene | 1.00 | 82.4 | 30.5 | 92.4 | 75.0 | 42.1 | 85.3 | 59.6 ± 26.7 |
| benzene | 6.09 | 121 | 137 | 79.8 | 57.4 | 85.8 | 39.4 | 86.7 ± 37.0 |
| 1,1,2-trichloroethane | 1.70 | 143 | 92.4 | 95.3 | 78.3 | 69.1 | 42.5 | 86.8 ± 33.5 |
| 1,1,2,2-tetrachloroethane | 1.61 | 75.0 | 60.2 | 61.7 | 51.1 | 54.5 | 10.8 | 52.2 ± 21.9 |
| 1,2-dichlorobenzene | 1.80 | 157 | 93.7 | 110 | 89.4 | 98.4 | 46.8 | 99.2 ± 35.6 |
| Mean | | | | | | | | 78.6 ± 33.5 |

Table 6-5. RECOVERY OF HALOGENATED HYDROCARBONS FROM DISTILLED WATER SPIKED FROM GAS MIXING BULB

| Compound | Amount spiked (μg) | % Recovery | | | | Average ± Std. Dev. |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | |
| methylene chloride | 1.33 | —[a] | —[a] | —[a] | —[a] | |
| chloroform | 1.48 | 88.5 | 73.7 | 47.9 | 55.0 | 66.3 ± 18.4 |
| bromodichloromethane | 1.98 | 98.1 | 121 | 84.4 | 85.8 | 97.3 ± 16.9 |
| tetrachloroethylene | 1.70 | 71.4 | 65.9 | 64.2 | 101 | 75.6 ± 17.2 |
| chlorobenzene | 1.11 | 81.7 | 81.7 | 79.7 | 89.7 | 83.2 ± 4.4 |
| m-dichlorobenzene | 1.35 | 82.3 | 110 | 68.5 | 102 | 90.7 ± 18.8 |
| Mean | | | | | | 79.2 ± 23.9 |

[a] Not quantifiable because of background interferences.

Table 6-6. RMR VALUES OF STANDARDS FOR 100 m CAPILLARY
ON THE LKB 2091 GC/MS

| Compound | MW | m/z | RMR |
|---|---|---|---|
| trichloroethylene | 130 | 95<br>130 | .79 ± 11%<br>.78 ± 13% |
| chloroform | 118 | 83 | 1.41 ± 8% |
| 1,2-dichloroethane | 98 | 62 | .431 ± 3% |
| 1,1,1-trichloroethane | 132 | 97 | 1.93 ± 10% |
| methylene chloride | 84 | 49<br>84 | .475 ± 5%<br>.294 ± 5% |
| tetrachloroethylene | 164 | 166 | .819 ± 3% |
| 1,1-dichloroethane | 98 | 63<br>65 | .646 ± 5%<br>.204 ± 7% |
| _m_-dichlorobenzene | 146 | 146 | .648 ± 7% |
| _o_-dichlorobenzene | 146 | 146 | .351 ± 17% |
| bromodichloromethane | 162 | 83<br>129 | 1.855 ± 13%<br>.302 ± 20% |
| carbon tetrachloride | 152 | 117<br>119 | 1.77 ± 7%<br>1.70 ± 7% |
| chlorobenzene | 112 | 112 | 1.82 ± 14% |

## VALIDATION OF PROCEDURES FOR WATER SAMPLES

### Sampling

Water samples are to be collected directly from the drinking water tap. This is a standard procedure and no problems were encountered in the preliminary sampling trips. No further validation was done.

### Purge Procedure

#### Radiolabeled Recovery Experiments --

The recoveries of radiolabeled model compounds from distilled water are shown in Table 6-7. The observed losses were attributed to leaks in the glassware. Breakthrough on the Tenax has been shown to be negligible in previous experiments (see "Efficiency and Reproducibility of Tenax Desorptions", above) and in validation of air sampling methods. Incomplete purging of the water was eliminated as a possibility by counting the residual radioactivity in the purged water samples. Less than 1% of the added radioactivity was found. Within the precision requirements of this study, the radiolabeled compound recoveries indicate the method is essentially quantitative.

#### Non-Radiolabeled Recovery Experiments --

The recoveries of five model compounds were determined as part of the experiments to evaluate spiking methods (see "Investigation of Methods for Loading Standards", above). These results are reported in Table 6-5.

In addition, a study was undertaken for determining the optimum purge time. The procedure involved spiking each of a number of distilled water samples with five halogenated hydrocarbons from a gas mixing bulb. These spiked samples were then purged with helium onto Tenax GC cartridges for times ranging from five minutes to ninety minutes. Duplicate loaded Tenax cartridges were then analyzed by FID and the percent recoveries calculated.

The gas mixing bulb for spiking water samples was prepared by heating to approximately 200°C and flushing with nitrogen (∿100 ml/min) for one hour. The temperature was then reduced to ∿70°C, the bulb sealed, and known amounts (approximately 2 μg) of chloroform, bromodichloromethane, tetrachloroethylene, chlorobenzene, and m-dichlorobenzene were injected. After equilibration for one hour, 1.0 ml aliquots were withdrawn with a gas-tight syringe and injected into 60 ml distilled water at -4°C contained

Table 6-7. RECOVERY OF $^{14}$C-LABELED MODEL COMPOUNDS FROM WATER[a]

| | Compound | | | |
|---|---|---|---|---|
| | Chloroform | Carbon tetrachloride | Chlorobenzene | Bromobenzene |
| 1 | 85.0 | 96.2 | 84.2 | 71.9 |
| 2 | 67.6 | 83.8 | 90.3 | 88.7 |
| 3 | 91.7 | 98.6 | 89.0 | 93.7 |
| 4 | 85.9 | 70.9 | 92.5 | 69.1 |
| 5 | 91.0 | 65.2 | 90.4 | 87.8 |
| 6 | 47.9 | 70.9 | 87.1 | 82.4 |
| 7 | 92.8 | 67.4 | 94.0 | 87.2 |
| 8 | 88.1 | - | 90.3 | 90.3 |
| Mean | 81.3 | 79.0 | 89.7 | 83.9 |
| ± S.D. | 15.7 | 14.0 | 3.1 | 8.9 |
| RSD (%) | 19.3 | 17.7 | 3.4 | 10.6 |

[a] 100 ml water spiked with 70,000-90,000 dpm of the model compound (~1 µg).
SD = standard deviation
RSD = (SD/Mean) x 100

in 60 ml amber bottles covered with septum caps. The gas aliquots were added to the distilled water one bubble at a time in order to allow maximum dissolution of the standard. After the standards were added to the distilled water, the samples were allowed to equilibrate for approximately 1 hr at ~4°C. In all, fourteen distilled water samples were prepared. Two distilled water blanks were also prepared. The spiked water samples and blanks were added to the purge flasks (Figure 6-1) and heated to 90°C. After the samples and the blank had reached 90°C, helium flow at 25 ml/min was begun. Purged compounds were collected on Tenax GC cartridges. Individual samples were purged for 5, 10, 15, 30, 45, 60 and 90 minutes. The loaded cartridges were desiccated overnight with anhydrous $CaSO_4$ in a Teflon-lined, screw cap culture tube.

Loaded Tenax cartridges were analyzed by thermal desorption GC/FID. Peaks were identified by comparison of retention times to individual standards. Recovery was determined by comparison of peak heights with calibration standards prepared by injecting 1 ml vapor aliquots from a gas mixing bulb directly onto Tenax GC cartridges.

The results of this analysis are presented in Table 6-8 and Figure 6-6. These results indicate that the recovery of all of the four halogenated hydrocarbons that could be quantified reaches a maximum at a purge time of approximately thirty minutes. Chloroform could not be quantified because of difficulty in peak identification.

### Summary

Based on the experiments summarized in Tables 6-5, 6-7 and 6-8, the expected recoveries of purgeable halogenated organics for water are about 80% or better. Within the precision requirements of this study, these recovery values indicate that the method is essentially quantitative. The entire analytical protocol has been tested during the preliminary field sampling studies. No significant problems are anticipated with execution of the protocol.

## VALIDATION OF PROCEDURE FOR BLOOD SAMPLES

### Sampling

Blood samples are collected in replicate 10 ml vacutainer tubes containing an anticoagulant. Using a qualified phlebotomist, the samples are

Table 6-8. PERCENT RECOVERY AT VARIOUS PURGE TIMES

| | | Purge Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Compound | Trial | 5 | 10 | 15 | 30 | 45 | 60 | 90 |
| Bromodichloromethane | I | [a] | 61.6 | [a] | 100.0 | [a] | 72.6 | 89.7 |
| | II | 66.1 | 106.8 | | 76.7 | 67.3 | 74.9 | 152.9 |
| Tetrachloroethylene | I | 14.2 | 29.7 | 74.7 | 80.7 | 77.3 | 73.8 | 70.4 |
| | II | 60.2 | 73.8 | | 65.1 | 59.7 | 55.4 | 41.1 |
| Chlorobenzene | I | 46.0 | 42.9 | 102.4 | 106.2 | 112.9 | 95.8 | 93.1 |
| | II | 71.8 | 58.9 | | 82.6 | 66.3 | 58.9 | 55.8 |
| *m*-Dichlorobenzene | I | 42.1 | 39.5 | 74.7 | 91.3 | 86.1 | 68.9 | 78.8 |
| | II | 32.4 | 38.4 | | 85.3 | 99.4 | 69.8 | 57.1 |
| Mean for all Compounds | | 51.3 | 75.1 | 83.9 | 86.0 | 79.5 | 71.3 | 79.9 |

[a] Interferences prevented quantification.

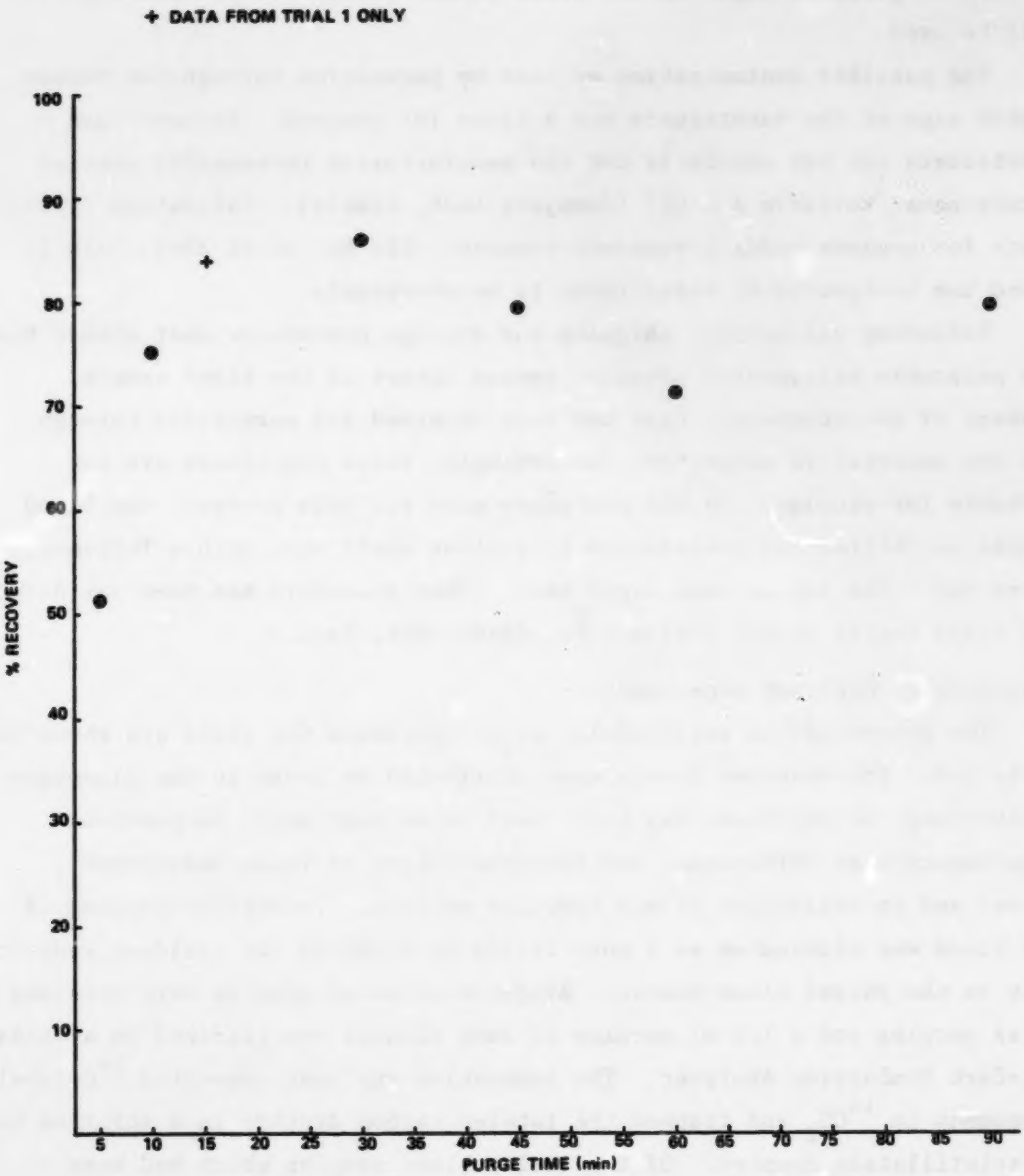

Figure 6-6. Percent recovery as a function of purge time.

collected by brachial venipuncture. Glass syringes represent the optimal collection device, since no polymeric material which may contaminate the sample comes in contact with the blood. However, sterilization of large numbers of glass syringes in the field is not practical, so vacutainers will be used.

The possible contamination or loss by permeation through the rubber septum caps of the vacutainers was a cause for concern. Teflon-lined vacutainers are not available but the manufacturers recommended special vacutainers "suitable for GC" (Venoject L428, Kimble). Validation experiments for benzene under a separate contract (EPA No. 68-01-3849, Task I) found the background of these tubes to be acceptable.

Following collection, shipping and storage procedures must assure that the purgeable halogenated organics remain intact in the blood sample. Leakage of the vacutainer caps has been observed and permeation through the cap material is suspected. Accordingly, these containers are not suitable for storage. In the procedure used for this project, the blood sample is chilled and transferred to a clean shell vial with a Teflon-lined screw cap. The cap is then taped shut. This procedure has been validated and field tested on EPA Contract No. 68-01-3849, Task I.

Radiolabeled Recovery Experiments--

The recoveries of radiolabeled model compounds for blood are shown in Table 6-9. The observed losses were attributed to leaks in the glassware. Breakthrough on the Tenax has been shown to be negligible in previous experiments (see "Efficiency and Reproducibility of Tenax Desorption", above) and in validation of air sampling methods. Incomplete purging of the blood was eliminated as a possibility by counting the residual radioactivity in the purged blood sample. Aliquots of blood samples were retained after purging and a 1.0 ml portion of each aliquot was oxidized in a Packard Tri-Carb Combustion Analyzer. The combustion analyzer converted $^{14}$C-labeled compounds to $^{14}CO_2$ and trapped the labeled carbon dioxide in a solution to be scintillation counted. Of the twelve blood samples which had been purged, only the samples which had been spiked with $^{14}$C-bromobenzene were radioactive. These three samples contained 7.7%, 12.7% and 4.9% (8.4% average) of the $^{14}$C-bromobenzene which had been added initially (77.1%,

Table 6-9. PERCENT RECOVERY OF $^{14}$C-LABELED COMPOUND FROM BLOOD[a]

| Compound | Mass Loading (µg) | dpm Loaded | % Recovery | Average % Recovery |
|---|---|---|---|---|
| chloroform | 1.46 | 91755.1 | 94.2 | 93.7 |
| | | | 93.1 | |
| | | | 46.5[b] | |
| carbon tetrachloride | 0.785 | 77902.3 | 92.6 | 89.4 |
| | | | 92.2 | |
| | | | 83.5 | |
| chlorobenzene | 1.12 | 89538.1 | 43.5[b] | 90.1 |
| | | | 88.0 | |
| | | | 92.2 | |
| bromobenzene | 1.21 | 84493.9 | 77.1 | 80.6 |
| | | | 74.4 | |
| | | | 90.3 | |

[a] Headspace purge of whole blood (25 ml) diluted 1:1 with distilled water and purged in 100 ml 3-neck purge flasks at 50°C for 90 min with helium at 25 ml/min.

[b] Leaking desorption chamber cap; not included in average.

74.4% and 90.3%, respectively, of the bromobenzene had been recovered on the Tenax GC cartridge). Since the boiling point of bromobenzene is 156°C, it seems reasonable that a headspace purge at 50°C may not completely remove this compound from the sample matrix.

Non-radiolabeled Recovery Experiments--

Blood was spiked with halogenated compounds by injecting a 1.0 ml aliquot of a vapor standard prepared in a gas mixing bulb into a septum-capped vial containing 25 ml of cold, outdated, whole human blood and 25 ml of distilled water. Aliquots (1.0 ml) of the vapor standards were also loaded onto Tenax GC cartridges. These cartridges served as quantitation standards. After allowing the spiked blood mixtures to equilibrate for 1 hr at 4°C, they were transferred to round-bottom three-necked flasks (100 ml capacity) topped with Tenax GC cartridges (Figure 6-2). The solution was heated to 50°C and the headspace above the liquid purged for 90 min at 25 ml He/min. Loaded cartridges were desiccated over $CaSO_4$ overnight and analyzed by GC/FID. The results are shown in Table 6-10.

### Summary

Based on the experiments summarized in Tables 6-9 and 6-10, the expected recoveries of purgeable halogenated organics are at least 80%. Within the precision requirements of this study, these recovery values indicate that the method is essentially quantitative. The entire analytical procedure has been tested during preliminary field sampling studies. No significant problems are anticipated with execution of the protocol.

## VALIDATION OF PROCEDURE FOR URINE SAMPLES

### Sampling

During the preliminary sampling trips, each participant was provided with a clean 120 ml (4 oz) bottle and asked to collect an early morning urine sample. In addition, spot urine samples were collected from selected participants at the time breath and blood samples were collected. No problems were encountered with this procedure and it will be followed in the future.

Table 6-10. RECOVERY OF HALOGENATED HYDROCARBONS FROM HUMAN BLOOD SPIKED FROM GAS MIXING BULB

| Compound | Amount spiked (µg) | % Recovery |  |  |  | Average ± Std. Dev. |
|---|---|---|---|---|---|---|
|  |  | 1 | 2 | 3 | 4 |  |
| methylene chloride | 7.96 | 105 | 116 | —[a] | —[a] | 111 ± 7.8 |
| chloroform | 8.16 | 84.6 | 145 | 139 | —[a] | 123 ± 33.1 |
| bromodichloromethane | 10.4 | 105 | 159 | 94.9 | 94.9 | 113 ± 30.7 |
| tetrachloroethylene | 8.52 | 108 | 121 | 82.8 | 97.5 | 99.8 ± 17.9 |
| chlorobenzene | 5.53 | 108 | 120 | 85.6 | 78.6 | 98.1 ± 19.3 |
| m-dichlorobenzene | 6.44 | 85.1 | 101 | 74.6 | 84.5 | 86.3 ± 10.9 |
| mean |  |  |  |  |  | 104.3 ± 22.7 |

[a] Not quantified because of background interferences.

### Purge Procedure

Radiolabeled Recovery Experiments --

The recoveries of radiolabeled model compounds from urine are shown in Table 6-11. The observed losses were attributed to leaks in the glassware. Breakthrough on the Tenax has been shown to be negligible in previous experiments (see "Efficiency and Reproducibility of Tenax Desorption", above) and in validation of air sampling methods. Incomplete purging of the water was eliminated as a possibility by counting the residual radioactivity in the purged water samples. Less than 1% of the added radioactivity was found. For $^{14}C$-bromobenzene, recoveries greater than 100% are attributed to quenching by methanol in the standard, reducing the dpm counted in the standards. Methanol quenching was not observed in the desorbed samples because of the low breakthrough volume for methanol on Tenax. Since the bromobenzene standards and samples were much more dilute, this quenching effect was more significant than for the other model compounds.

Non-Radiolabeled Recovery Experiments --

The recoveries of six model compounds from urine are summarized in Table 6-12.

### Summary

Based on the experiments summarized in Tables 6-11 and 6-12, the expected recoveries of purgeable halogenated organics are about 80%. Within the precision requirements of this study, these recovery values indicate that the method is essentially quantitative. The entire analytical procedure has been tested during the preliminary field sampling studies. No significant problems are anticipated with execution of the protocol.

## VALIDATION PROCEDURE FOR TISSUE SAMPLES

### Sampling

It is anticipated that tissue samples collected from cadavers or surgery will be obtained from a pathologist. Personnel from RTI will work with pathologists advising them of proper sample handling procedures. To be of use for purgeable halogenated organics a tissue sample must be collected a short time following death and immediately frozen in a cleaned glass container with as small a "headspace" as possible. Any handling or

Table 6-11. PERCENT RECOVERY OF $^{14}$C-LABELED COMPOUNDS FROM URINE

| Compound | Mass Loaded (µg) | dpm Loaded | % Recovery | Average % Recovery |
|---|---|---|---|---|
| chloroform | 1.50 | 94237.0 | 83.9<br>84.3<br>85.2 | 84.5 |
| carbon tetrachloride | 0.809 | 80278.6 | 57.2<br>66.7<br>67.1 | 63.7 |
| chlorobenzene | 1.12 | 89589.9 | 89.9<br>87.8<br>90.2 | 89.3 |
| bromobenzene | 0.0127 | 885.4 | 147<br>114<br>107 | 123 |

### Table 6-12. RECOVERY OF HALOGENATED HYDROCARBONS FROM HUMAN URINE SPIKED FROM GAS MIXING BULB

| Compound | Amount spiked (μg) | % Recovery | | | | Average ± Std. Dev. |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | |
| methylene chloride | 6.96 | 80.6 | —[a] | 41.3 | 21.7 | 47.9 ± 30.0 |
| chloroform | 8.16 | 107 | 72.6 | 89.5 | 44.8 | 78.5 ± 26.5 |
| bromodichloromethane | 10.2 | 123 | 88.2 | 126 | 80.8 | 105 ± 23.3 |
| tetrachloroethylene | 8.44 | 85.2 | 61.2 | 84.4 | 57.1 | 72.0 ± 14.9 |
| chlorobenzene | 5.58 | 103 | 77.1 | 92.6 | 71.9 | 86.2 ± 14.2 |
| *m*-dichlorobenzene | 7.54 | 73.6 | 70.5 | 90.1 | 80.7 | 78.7 ± 8.7 |
| mean | | | | | | 79.2 ± 24.3 |

[a] Not quantified because of background interferences.

storage in contact with polymeric materials represents potential contamination.

Samples received from Niagara Falls, New York during the preliminary sampling period were not handled as above and were useless for purgeable halogenated organics analysis. Among the improper handling procedures were: storage in plastic bags, treatment with formalin, refrigeration (not freezing) of the samples for at least one week postmortem, and thawing of the sample to cut off portions for shipment to RTI.

## Purge Procedure

### Radiolabeled Recovery Experiments --

No radiolabeled recovery experiments were conducted with tissue.

### Non-radiolabeled Recovery Experiments --

Human adipose tissue specimens were obtained from pathology departments of local hospitals, fortified with halogenated compounds and purged to assess recovery. Samples (5 g) of frozen human fat were cut from a larger mass with a scalpel, sectioned and transferred to a 100 ml, round-bottom, three-necked flask. Aliquots of distilled water (60 ml) were fortified with 1 μg each of methylene dichloride, chloroform, bromodichloromethane, tetrachloroethylene, chlorobenzene and m-dichlorobenzene using a gas mixing bulb. The water was added to the tissue in the purge flask and the mixture macerated in an ice bath using a Virtis tissue homogenizer. The purge apparatus was immediately assembled, heated to 50°C and the headspace purged for 30 minutes at 25 ml He/min. The exposed cartridges were desiccated and analyzed by GC/FID as described in the "Materials and Methods" section.

The recovery of halogenated hydrocarbons from human adipose tissue (Table 6-13) vary significantly. Great difficulty was encountered in quantitative introduction of a representative fortified sample into the container for analysis. Consequently variations in recovery may be attributed to losses during tissue maceration and transfer.

## Summary

The tissue sample analysis procedure has been partially validated. Due to some low recovery values listed in Table 6-13, the difficulties in handling tissue during the purge, and (most significantly) the lack of control over

## Table 6-13. RECOVERY OF HALOGENATED HYDROCARBONS FROM HUMAN ADIPOSE TISSUE

| | % Recovery | | | | |
|---|---|---|---|---|---|
| Compound | 1 | 2 | 3 | 4 | Average + S.D. |
| methylene chloride | ——[a] | 78.2 | 69.8 | 92.8 | 80.3 ± 11.6 |
| chloroform | ——[a] | 62.3 | 14.8 | 59.7 | 45.6 ± 26.7 |
| bromodichloromethane | 64.4 | 15.5 | 13.4 | 49.4 | 35.7 ± 25.3 |
| tetrachloroethylene | 39.4 | 15.2 | 112 | 40.6 | 51.8 ± 41.8 |
| chlorobenzene | 21.5 | 6.8 | 7.3 | 17.9 | 13.4 ± 7.5 |
| *m*-dichlorobenzene | 82.1 | ——[a] | 56.5 | 30.8 | 56.5 ± 25.7 |
| mean | | | | | 45.3 ± 30.8 |

[a] Not quantified due to peak interference.

sampling and storage prior to receipt by RTI; the analysis of tissue samples of a purgeable halogenated organics must be regarded as semi-quantitative.

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## SECTION 7

## COLLECTION AND ANALYSIS OF "EXTRACTABLE" HALOGENATED ORGANICS IN ENVIRONMENTAL AND HUMAN SAMPLES

The "extractable" halogenated organics, also referred to as "semi-volatiles" and "pesticides and PCBs" are those compounds which may be solvent-extracted and concentrated for analysis. At the lower end, volatile compounds such as tetrachloroethylene, chlorobenzene and lighter compounds are either lost in solvent evaporation and/or are not chromatographically separated from the solvent peak. At the upper end, nonvolatile (high boiling) and/or polar compounds do not elute from the chromatography column under the conditions to be used. Compounds which would not elute include decabromobiphenyl and tris(2,3-dibromopropyl)phosphate.

The compounds of interest in this class include halogenated aromatics, halogenated pesticides, and PCBs. Their polarity, volatility and reactivity all make them amenable to standard pesticides residue methods. Therefore, the collection, extraction, cleanup, concentration, and analysis procedures are modifications of these standard methods. Briefly, semi-volatile halogenated hydrocarbons are extracted from the matrix with organic solvents, dried, concentrated, and, where necessary, subjected to chromatographic cleanup on Florisil to remove lipids and other non-gas chromatographable impurities. The sample is analyzed by GC/ECD. Qualitative identifications are based upon retention times using two columns and quantification is achieved by comparison of detector response to that of standards. Identifications are confirmed by GC/MS/COMP when sufficiently concentrated.

### GENERAL METHOD VALIDATION

### Materials and Methods

The pesticides and PCB mixtures (Aroclor®) used were obtained from the Pesticides Repository of the Quality Assurance Section, Analytical Chemistry

Branch, EPA, HERL, ETD (MD-69), Research Triangle Park, NC 27711. Individual PCB isomers were obtained from RFR Corp., Hope, RI, and $d_{10}$-pyrene from Merck and Co., Inc., Isotopes, St. Louis, MO. All solvents were "Distilled in Glass" (Burdick and Jackson, Muskegon, MI) and redistilled prior to use.

Extraction and cleanup validation samples were analyzed using a Fisher/-Victoreen 4400 gas chromatograph equipped with a microcell electron capture detector (GC/ECD). The primary analytical column was a 0.38 mm i.d. x 38 m 1% SE-30, 0.32% Tullanox SCOT capillary column. Operating conditions were: column temperature, 220°C; injection temperature, 265°C; detector temperature, 285°C; carrier gas, 2.5 ml/min nitrogen, with nitrogen makeup gas adjusted to a total flow of 25 ml/min. A column used to verify extractable halogenated organics in samples at concentrations below the GC/MS/COMP detection limit was a 180 cm x 2.0 mm i.d. glass column packed with 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W(HP). The operating conditions were: column temperature, 220°C isothermal; detector temperature, 285°C; injector temperature, 265°C; carrier gas 18 ml/min nitrogen.

The GC/MS/COMP systems used were a Finnigan 3300 GC/MS/COMP and an LKB 2091 GC/MS equipped with an LKB 2031 data system. Operating conditions for the Finnigan 3300 were: 20 m x 0.38 mm i.d., 1% SE-30 SCOT capillary operated isothermally at 235°C and a flow rate of 2.5 ml/min helium. Splitless injection (0.2 - 0.3 µl) was used, with standard electron impact (70 eV) ionization conditions.

The LKB 2091 was operated using a 18 m 1% SE-30/$BaCO_3$ SCOT capillary column at 240°, isothermal for PCBs and a 40 m x 0.38 mm i.d. 1% SE-30 SCOT capillary column at 230° isothermal for the pesticides. In both cases, the column flow rate was 2 ml/min with 20 ml/min split off at the injector. The mass spectrometer was operated under standard electron impact conditions as described in Section 6.

The Finnigan 3300 and the LKB 2091 systems were operated in both the full scan and single ion monitoring (SIM) modes. In the full scan mode spectra are repetitively collected. Spectra or mass fragmentograms (single ion plots) may be plotted for interpretation. In the SIM mode, only a small number (up to 9 for the Finnigan 3300 and up to 16 for the LKB 2091) of ions are monitored. Full spectra are not collected. The advantage of this method is that the detector spends more time "looking" at the selected ions and therefore better (generally 10-50 times) sensitivity is obtained.

## Validation of Gas Chromatographic Procedures

Due to the selectivity and sensitivity of GC/ECD, this will be the primary procedure for analysis of extractable halogenated organics. Identifications by GC/ECD will be confirmed on a second chromatographic column and, where the sample concentration is sufficient, by GC/MS/COMP.

Using both the SE-30 SCOT capillary column and the OV-17/QF-1 packed column, retention times were determined for the standards used in recovery studies (*vide infra*) and others as needed. Using the operating conditions appropriate for pesticides analysis, the linear response of the ECD was found to be from about 186 to <2 pg for trifluralin and 220 to <2 pg for lindane. For the purposes of routine analysis, an upper detection limit of 160 pg will be asssumed.

## Validation of GC/MS/COMP Analysis Procedures

The chromatography conditions were similar to those used for GC/ECD, The samples for this study to be screened by GC/ECD were to be confirmed (if sufficiently concentrated) by GC/MS/COMP. Therefore, the retention times of the two techniques must be similar - GC/ECD must operate isothermally, so the GC/MS/COMP conditions reflect this restriction.

The basic areas of validation for the GC/MS/COMP analysis procedure were to determine conditions which provide the lowest limit of detection (LOD) and to determine Relative Molar Response (RMR) factors for compounds of interest to permit quantitation in samples.

### Determination of Limits of Detection for GC/MS/COMP --

Standard solutions of selected pesticides and PCB isomers were analyzed on the Finnigan 3300 and LKB 2091. In the full scan mode the limit of detection was the amount of compound required for an interpretable spectra. In the SIM mode, the limit of detection was the amount of compound required to yield a peak 2-4 times the noise level. The estimated limits of detection for the Finnigan 3300 and LKB 2091 are presented in Table 7-1.

Table 7-1. ESTIMATED LIMITS OF DETECTION FOR EXTRACTABLE HALOGENATED ORGANIC ANALYSIS[a]

| Compound | LKB 2091[b] | | | Finnigan 3300[c] | | |
|---|---|---|---|---|---|---|
| | Full Scan ng/μl | SIM | | Full Scan ng/μl | SIM | |
| | | m/z | ng/μl | | m/z | ng/μl |
| trifluralin | 12 | 264 | 0.4 | 5-10 | 264 | <0.5 |
| atrazine | >12<20 | 200 | 0.4 | <50 | 200 | 5-10 |
| γ-BHC (lindane) | >12<20 | 181 | 0.18-0.4 | 5-10 | 181 | 1 |
| heptachlor | 12 | 272 | 0.18-0.4 | 10-20 | 272 | 1-1.5 |
| chlordane | ∿30[c] | 375 | 5 | 25-50 | 375 | 5-10 |
| *p*,*p*'-DDE | 12 | 246 | >0.3 | 5-10 | 246 | 0.5-1 |
| 2-chlorobiphenyl | ∿1 | 188 | 0.004 | ∿2.5 | 188 | ∿0.025 |
| hexachlorobiphenyl | <1 | 360 | ∿0.016 | 25-50 | 360 | ∿0.15 |
| decachlorobiphenyl | 12 | 498 | 0.42 | 150 | 498 | ∿0.3 |

[a] See text for conditions.

[b] 15:1 split at injector, gc only 1/15 of injection is on column.

[c] 0.2 μl injected with no split.

Representative mass spectra, single ion plots and reconstructed gas chromatograms are included in Appendix L to illustrate the limits of detection.

Determination of Relative Molar Response Factors (RMRs) for GC/MS/COMP--

Tables 7-2 and 7-3 present the RMRs for PCBs and pesticides determined on the LKB-2091 system. The high limits of error for the early eluting components (through lindane) are due to the fact that only three scans were acquired across the peak (7.2 sec wide peaks). Attempts were made to degrade the performance of the column by lowering the column temperature or by lowering the flow rate. This, however, led to poor intensities for the later eluting components without increasing the peak width significantly.

Typical single ion plots used for the RMR determinations are presented in Appendix M.

## VALIDATION OF PROCEDURES FOR BLOOD

The procedure to be used to work up the extractable halogenated organics in blood was modified from that of Thompson (7-1). Two major validation efforts were: determination of whether whole blood or plasma should be extracted and validation of the Florisil cleanup procedure. These efforts are discussed below.

### Validation of Extraction Procedure

During the validation procedure it was determined that no pesticides were extractable from the cells. This indicated that only the plasma fraction which is much easier to work with than whole blood, need be analyzed. Briefly, when whole blood was spiked with pesticides, recoveries from the plasma fraction were variable and low and almost no pesticide was found in the cells. When plasma was spiked, recoveries were both higher and less variable. These results are summarized in Table 7-4. However, with longer equilibration (19 hr vs 2 hr), as shown in Table 7-5, the recoveries from plasma were significantly lower. The paragraphs below describe the validation experiments in detail.

Eight pesticides at the 500 ng concentration level were added to four 7 ml aliquots of whole blood. Each tube was incubated at 37°C for two hours and centrifuged for ten minutes. The plasma was separated from whole blood cells by pipetting off all possible plasma and submitting the whole cell

Table 7-2. RMRs FOR PCBs AND PESTICIDES OF INTEREST TO THIS PROGRAM[a]

| Compound | Concentration | Ion | RMR |
|---|---|---|---|
| 2-chlorobiphenyl | 104 ng/µl | 188 | elutes with solvent and was two scans wide - not determinable |
|  | 3.8 ng/µl |  |  |
| hexachlorobiphenyl | 570 ng/µl | 360 | .38 ± 3% |
|  | 10.4 ng/µl |  | .35 ± 10% |
| decachlorobiphenyl | 1156 ng/µl | 498 | .14 ± 7% |
|  | 8.4 ng/µl |  | not determinable |
| trifluralin | 100 ng/µl | 264 | 1.32 ± 20% |
| atrazine | 100 ng/µl | 200 | .74 ± 7% |
|  |  | 202 | .25 ± 8% |
| lindane | 100 ng/µl | 181 | .74 ± 9% |
|  |  | 183 | .62 ± 12% |
| heptachlor | 100 ng/µl | 272 | .74 ± 6% |
| *p*,*p*'-DDE | 100 ng/µl | 246 | .45 ± 6% |
| chlordane (peak 1) | 100 ng/µl | 373 | .71 ± 5% |
|  |  | 375 | .65 ± 5% |
| chlordane (peak 2) | 100 ng/µl | 373 | .051 ± 6% |
|  |  | 375 | .045 ± 13% |

[a] Standard is $d_{10}$-pyrene (m/z = 212)

Table 7-3. RMR FACTORS FOR STANDARD PCB SOLUTIONS, SELECTED ION MONITORING MODE[a]

| Standard | RMR m/z 188 2-Chlorobiphenyl | | RMR m/z 358 Hexachlorobiphenyl | | RMR m/z 498 Decachlorobiphenyl | |
|---|---|---|---|---|---|---|
| I PCB-STD-20 | 0.60 | | 0.257 | | 0.341 | |
| II PCB-STD-2 | 0.620<br>0.811<br>0.466<br>0.643 | 0.640 ± .171 | 0.291<br>0.334<br>0.319<br>0.321 | 0.325 ± .009 | 0.430<br>0.474<br>0.462<br>0.431 | 0.456 ± .018 |
| III PCB-STD-0.2 | 0.566<br>0.840<br>0.637<br>0.597<br>0.705 | 0.699 +.171 | 0.366<br>0.293<br>0.301<br>0.239<br>0.273 | 0.294 ± .072 | 0.372<br>0.361<br>0.373<br>0.303<br>0.394 | 0.361 ± .033 |
| IV PCB-STD-0.04 | 1.020<br>0.692<br>0.576 | 0.763 ± .257 | 0.320<br>0.528<br>0.528 | 0.459 ± .072 | 0.287<br>0.543<br>0.372 | 0.401 ± .142 |

[a] Standard is $d_{10}$-pyrene (m/z = 212)

Table 7-4. RECOVERY OF EXTRACTABLE HALOGENATED ORGANICS FROM WHOLE BLOOD AND PLASMA

| | Percentage Recoveries ± Std. Dev. | | | |
|---|---|---|---|---|
| | Whole Blood (∿500 ng spike) | | | |
| Compound | Plasma[a] | Cell[b] | Plasma[c] ∿500 ng spike | Plasma[d] ∿10 ng spike |
| Trifluralin | 36.0 ± 7.5 | Not det. | 87.3 ± 7.7 | -[e] |
| Lindane | 62.8 ± 12.7 | <1.0 | 106.8 ± 4.9 | 100.6 ± 9.2 |
| Heptachlor | 29.0 ± 9.0 | Not det. | 106.3 ± 8.1 | 88.5 ± 5.0[f] |
| Heptachlor epoxide | 31.3 ± 7.6 | Not det. | 63.1 ± 1.4[g] | 59.4 ± 11.6 |
| Endosulfan | 37.7 ± 8.5 | Not det. | 75.0 ± 4.3 | 73.4 ± 5.4 |
| Dieldrin | 31.6 ± 10.4 | Not det. | 79.6 ± 4.2 | 79.6 ± 6.4 |
| p,p'-DDT | 82.9 ± 24.8 | Not det. | 101.4 ± 15.8[h] | 78.8 ± 10.1[i] |
| Mean | 44.4 ± 20.4 | <1 | 88.5 ± 17.0 | 80.1 ± 13.9 |

[a] Data based on mean of four samples, each of which is quantified twice against a standard. Whole blood was spiked and separated. Extractions were for a short time (∿45 min).

[b] Based on injection of two of four samples--Not Det. = not detected.

[c] Data based on mean of three samples, each of which is quantified twice against a standard. Whole blood spiked; plasma fraction extracted over long time (∿24 hr).

[d] Plasma spiked ane extracted with hexane 24 hr, then 12 hr with hexane. Data based on mean of two samples, each of which is twice quantified against a standard.

[e] Compound elutes with solvent; not quantified.

[f] Based only on standard deviation of standard.

[g] Data based only on one sample. Precision between samples very poor.

[h] 47 ng spiked.

[i] Based only on standard deviation of sample.

## Table 7-5. RECOVERY STUDIES OF EXTRACTABLE HALOGENATED HYDROCARBONS FROM HUMAN PLASMA EQUILIBRATED FOR 19 HR

| | | Recovery from Plasma (%)[a] | | | |
|---|---|---|---|---|---|
| Compound | ng spiked | Trial One | Trial Two | Trial Three | Mean |
| Trifluralin | 14.8 | 87.6 | 92.3 | 83.3 | 85.7 |
| α-BHC | 14.0 | 61.9 | 56.2 | 57.6 | 58.6 |
| β-BHC | 14.5 | 60.0 | 60.1 | 43.8 | 50.8 |
| γ-BHC | 15.2 | 57.1 | 53.3 | 52.4 | 54.3 |
| 2,4,5-Trichlorobiphenyl | 14.0 | 48.2 | b | b | 48.2 |
| Heptachlor | 13.6 | 50.2 | 42.2 | 51.1 | 47.8 |
| Aldrin | 14.8 | b | 40.3 | 37.2 | 38.8 |
| Heptachlor Epoxide | 14.2 | 35.2 | 43.1 | b | 39.2 |
| Endosulfan | 13.1 | 53.2 | 59.1 | 44.3 | 52.2 |
| p,p'-DDE | 11.6 | 51.4 | b | 48.8 | 50.1 |
| Dieldrin | 15.8 | 58.6 | b | 58.5 | 58.6 |
| Mean ± Std. Dev. | | | | | 53.1 ± 12.6 |

[a] Experimental conditions similar to those described in Footnote d, Table 7-1, except spiked plasma was equilibrated 19 hr *vs.* 2 hr.

[b] Not quantifiable due to instrumental problems.

fraction to four successive 0.85% NaCl washes. Layers were again separated by centrifugation. Each fraction was successively extracted with 4 ml of hexane for 45 minutes; a 4 ml aliquot was then removed, 4 ml of fresh hexane added, extraction continued for 45 minutes, and another 4 ml aliquot of hexane removed. The samples were then dried over anhydrous $Na_2SO_4$, concentrated to the proper linear response range, and quantified by GC/ECD. Table 7-4 shows the partitioning of pesticides between whole blood and plasma. Virtually no detectable pesticides were detected in whole cell extracts, and recoveries from plasma were poor.

Eight pesticides at the 500 ng concentration level were added to three 2 ml aliquots of human plasma. Each tube was incubated at 37°C for two hours and extracted with 7.0 ml hexane for 24 hours. A 6.0 ml aliquot of solvent was removed from each, 6.0 ml fresh hexane was added, extraction continued 12 hours, and 6.0 ml again removed. Each sample was dried over $Na_2SO_4$, concentrated to the proper linear response range, and analyzed by GC/ECD. Table 7-4 shows the percentage recovery by this methodology.

The experiment above was repeated at spiking levels near the limit of detection (about 10 ng) to determine if the recovery is concentration-dependent. The results presented in Table 7-4 indicate that good recoveries are achieved even at low limits.

The recoveries were again determined with a longer equilibration time (19 hr) at a 11-16 ng spiking level. All other conditions were the same. The recoveries, shown in Table 7-5, are significantly lower than the analogous data in Table 7-4 (mean 53.1% vs 80.1%). These data indicate that the model compounds may be binding to the plasma matrix to form unextractable complexes.

### Summary

Based on the experiments summarized in Table 7-4 and 7-5, the expected recoveries of extractable halogenated organics from blood are about 40-100%. Because of the apparent low recoveries, this method must be regarded as semi-quantitative.

## VALIDATION OF PROCEDURE FOR WATER

The water solubilities of most of the compounds of interest are very low and the probability of finding any extractable halogenated organics in water is remote. Nevertheless, an extraction procedure was validated for water samples so that at least some of the samples may be run to confirm this assumption.

The recoveries of selected pesticides from water, listed in Table 7-6, indicate that the procedure is, in general, satisfactory. These pesticides were chosen as representative of the range of polarity and volatility expected in the compounds to be studied.

Based on the experiments summarized in Table 7-6, the expected recoveries of extractable halogenated organics from water are about 70% or better. Within the precision requirements of this study, these recovery values indicate that the method is essentially quantitative. The entire analytical procedure has been tested during preliminary field sampling studies. No significant problems are anticipated with the execution of the protocol.

## VALIDATION OF PROCEDURE FOR TISSUE

The procedure for tissue followed that of Thompson (7-1) with minor modifications. Recovery studies were done on human samples received from the Buffalo/Niagara Falls area. The recoveries, listed in Table 7-7 indicate that the method works not only for adipose tissue (for which it was designed) but also for other tissue samples. The paragraphs below detail the analytical procedure and the rationale behind the individual steps.

Tissue fractions were analyzed for semi-volatile halogenated hydrocarbons using a modified procedure by Thompson (7-1). Approximately five grams of each tissue was ground in a large glass beaker with a glass rod with acid-washed, sea sand and sodium sulfate (both cleaned up prior to use by extraction in a Soxhlet extractor with hexane) until a dry, granular mass was obtained. Aldrin, 198 ng in 100 µl hexane was added to the sample as an internal standard. Each tissue was then extracted, with vigorous grinding, with three 50 ml aliquots of hexane for approximately 5 min each. The extracts were then filtered, concentrated in a Kuderna-Danish (KD) apparatus to approximately 10 ml, blown down under nitrogen to dryness at room temperature and weighed.

The high lipid content of the adipose tissue extract necessitated partitioning of the extract into acetonitrile and back-partitioning of the halogenated hydrocarbons into hexane. Approximately 2.5 grams of fat extract

Table 7-6. RECOVERY STUDIES OF EXTRACTABLE HALOGENATED HYDROCARBONS FROM WATER

| Compound | Recovery (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Low Fortification[a] | | | High Fortification[b] | | | |
| | Trial One | Trial Two | Average | Trial One | Trial Two | Trial Three | Average |
| Trifluralin | 55.4 | 48.1 | 51.8 | 68.3 ± 0.8 | 69.9 ± 4.3 | 126.8 ± 14.0 | 88.3 ± 11.2 |
| α-BHC | 57.8 | 57.9 | 57.8 | 93.2 ± 5.0 | 98.7 ± 6.2 | 82.2 ± 1.2 | 91.4 ± 7.7 |
| β-BHC | 54.7 | 65.1 | 65.1 | –[c] | – | – | – |
| γ-BHC | 65.6 | 70.6 | 68.1 | – | – | – | – |
| 2,4,5-Trichlorobiphenyl | 62.4 | 73.1 | 67.8 | – | – | – | – |
| Heptachlor | 99.1 | 93.6 | 96.4 | 71.0 ± 6.0 | 66.4 ± 4.3 | 83.8 ± 2.0 | 73.7 ± 8.0 |
| Aldrin | 58.3 | 47.8 | 53.0 | – | – | – | – |
| Heptachlor epoxide | 87.4 | 76.1 | 81.8 | – | – | – | – |
| Endosulfan | 78.7 | d | 78.7 | 81.1 ± 1.4[e] | 103.1 ± 8.7 | 90.9 ± 7.9 | 91.7 ± 11.2 |
| p,p'-DDE | 73.8 | 80.3 | 77.0 | – | – | – | – |
| Dieldrin | 82.0 | 88.6 | 85.3 | 57.5 ± 4.7 | 67.9 ± 8.7 | 63.5 ± 4.9 | 63.0 ± 10.7 |
| Mean ± Std. Dev. | | | 70.7 ± 14.4 | | | | 74.0 ± 21.7 |

[a] 100 ml distilled/deionized water fortified with 11-16 ng of model compound in 100 μl methanol, extracted according to protocol.
[b] Same as (a) except fortification level about 500 ng.
[c] Not included in this experiment.
[d] Not quantifiable due to instrumental problems.
[e] Only one standard injection.

Table 7-7. RECOVERY OF EXTRACTABLE HALOGENATED HYDROCARBONS FROM HUMAN TISSUE EXTRACTS

| Tissue | Percent Extractable Material[a] | Percent Recovery[b] |
|---|---|---|
| Adipose[c] | 84.3 | 76.6 |
| Brain | 4.4 | 69.7 |
| Liver | 1.1 | 85.1 |
| Kidney | 1.1 | 64.5 |
| Spleen | 1.3 | 91.2 |
| Lung | 0.4 | 87.5 |

[a] Also described as "percent fat" in some procedures.

[b] Recovery of 198 ng aldrin added to tissues after maceration and before extraction as an internal standard. Percent recovery determined after all analytical manipulations. Aldrin represents a suitable standard, since it is metabolized to endrin and has not been found in tissues. Mean recovery for adipose tissues = 79.1 ± 10.6%.

[c] Analytical workup includes acetonitrile partitioning to reduce fat content.

was dissolved in 12 ml hexane and extracted two min each with four 30 ml aliquots of redistilled acetonitrile. The extracts were combined with 250 ml 2% aqueous sodium chloride and back-extracted with four 30 ml aliquots of hexane. The hexane extracts were combined, dried over $Na_2SO_4$, concentrated in a KD to about 5 ml, blown down under nitrogen to approximately 2 ml, and subjected to Florisil cleanup as described below.

Florisil (60/100 mesh, activated at 130°C overnight) columns (2.2 cm i.d. x 10 cm) with glass frits or glass wool plugs were packed in hexane solvent. Each tissue extract was transferred to the surface of the column in approximately 2 ml of solvent, and the column walls were washed with approximately 4 ml of hexane. The halogenated hydrocarbons were then eluted with 200 ml each of 6% ether/hexane and 15% ether/hexane, respectively. Each fraction was concentrated in a KD apparatus to approximately 5 ml.

After storage in the refrigerator overnight, a precipitate was found in the concentrated 15% eluant of the adipose and brain extracts. Consequently, these fractions were stored in the freezer prior to further cleanup and analysis. The 6% eluants were blown down under ambient nitrogen to appropriate volumes and analyzed by GC/ECD.

Previous studies by Thompson suggest the following halogenated hydrocarbons should be found in the 6% eluant: BHC isomers, p,p'-DDE, p,p'-DDT, heptachlor, heptachlor epoxide, mirex, PCB, hexachlorobenzene, and trifluralin (7-1). These represent the same general polarity of the halogenated compounds of interest in tissue samples, so this fraction is of primary interest for analysis.

Based on the experiments summarized in Table 7-7, the expected recoveries of extractable halogenated organics from tissue are 60-90%. This procedure has only been partially validated so, depending upon the quality control studies carried out with field samples, the data may need to be corrected for low recoveries. The entire analytical procedure has been tested during preliminary field sampling studies. No significant problems are anticipated with the execution of the protocol.

## VALIDATION OF PROCEDURE FOR URINE

Based on literature data (7-2) the concentrations of most of the extractable halogenated organics of interest is very low. Therefore, significant results are not expected from urine samples. The procedure to be used, described by Thompson (7-1) has not been validated. Quality control samples analyzed with the field samples will serve to determine recoveries. If recoveries are less than quantitative, correction factors will be used.

## VALIDATION OF PROCEDURE FOR SOIL AND SEDIMENT

The procedure to be used, described in Thompson (7-1) has not been validated. Quality control samples analyzed with the field samples will serve to determine the recoveries. If recoveries are less than quantitative, correction factors will be used.

## REFERENCES

7-1 Thompson, J. F., Analysis of Pesticide Residues in Human and Environmental Samples, A Compilation of Methods Selected for Use in Pesticide Monitoring Programs, Environ. Toxicol. Div., Health Effects Research Lab., USEPA, RTP, NC, December, 1974.

7-2 Pellizzari, E. D., MTPR No. 15, EPA Contract No. 68-01-4731, February 1979, Appendix B.

## SECTION 8

## COLLECTION AND ANALYSIS OF BLOOD SAMPLES FOR CEA

The selection of the CEA-Roche radioimmunoassay (RIA) was in part predicated on the large body of data on normal and abnormal populations in this test (8-1 - 8-4). However the validation of the accuracy of the test must be performed in each laboratory with standards. In order to test the overall procedure from sample collection to data analysis the CEA analysis (Appendix J) was performed on the 9 participants in the "Love Canal" pilot study. The results of the analysis of these participants in the pilot study are given in Table 8-1. The CEA-Roche procedure indicates standard deviations are roughly ± 0.5 ng/ml in the 0 to 5 ng/ml range.

CEA levels are not considered elevated until they exceed 2.5 ng/ml by this procedure. Three individuals in this group fall into that category. In a normal healthy population of non-smokers the predicted frequency of elevated CEA is 3 in 100 and 19 per 100 for smokers. Of the 9 participants in this study, 5 were smokers. By using a weighted average incidence of elevated CEA levels for smokers and non-smokers, the 3 elevated CEA levels in this pilot was of borderline significance (barely significant at the 90% confidence level). This is a very small population to try to analyze statistically, but this indicates the approach to be taken.

### REFERENCES

8-1 Go VLW: Data on file, Hoffmann-La Roche Inc., Nutley, NJ.
8-2 Hansen, H. J., et al., J. Clin. Res., 19, 143 (1971).
8-3 Data available on request from Hoffmann-La Roche Inc., Nutley, NJ.
8-4 Hansen, H. J., et al., Human Pathology, 5, 139-147 (1974).

Table 8-1. PLASMA CEA LEVELS OF INDIVIDUALS RESIDING IN THE "OLD LOVE" CANAL SITE OF NIAGARA FALLS, NY

| Sample No. | Mean ng/ml | Standard Error[a] ng/ml |
|---|---|---|
| 10009 | 1.91 | 0.30 |
| 10017 | 1.18 | 0.21 |
| 10025 | 1.11 | 0.66 |
| 10033 | 2.29 | 0.27 |
| 10066 | 1.40 | 0.46 |
| 10074 | 1.98 | 0.21 |
| 10041 | 3.02[b] | 0.69 |
| 10058 | 2.74[b] | 0.50 |
| 10090 | 2.58[c] | - |

[a] The standard error was computed from $(s^2_{\text{standards}} + s^2_{\text{samples}}/n)^{1/2}$.

[b] Initial assay resulted in loss of one sample. A separate assay was made after the expiration date of the kit.

[c] Initial assay resulted in loss of one sample and insufficient plasma remained for a repeat.

## SECTION 9

## COLLECTION AND ANALYSIS OF HALOGENATED ORGANICS IN HUMAN BREATH

The apparatus used for the collection of halogenated organics was developed under Contract No. 68-01-3849 for the analysis of benzene in human breath. The performance of the sampling apparatus was validated for benzene in a pilot study using filling station attendants, fuel truck drivers and laboratory personnel. These validation experiments with benzene serve as a model for the volatile halogenated hydrocarbons. Individuals for both work and non-work exposures, where the responses of the breath analysis to exposure could be estimated. In the case of benzene, smoking constitutes a substantial exposure.

It was our original intent to use all non-smokers. However, of the relatively limited population available to us for this pilot study, a very high percentage of them were smokers. We were, therefore, only able to obtain participation of one non-smoker filling station attendant.

Each participant was supplied with a personnel sampler and a Tenax cartridge 6-8 hours preceding the breath sample collection. A blood sample was drawn (~10 ml) and the breath sample collected immediately thereafter. All of the work exposure samples were collected on a Friday afternoon after a minimum of five consecutive days of working 10-12 hours per day. The off-day sampling was performed either on Sunday afternoon or before work on Monday morning. In the case of participant E, there was a 2-week delay between the Friday of the work-day sample and Sunday off-day sample. Otherwise, the sampling was done on consecutive Fridays and Sundays. In order to obtain baseline information, two non-smoking laboratories workers were sampled on their work days.

Table 9-1 summarizes the results from the analysis of all of the participants in the breath analysis. The breath sampling followed the protocol for breath analysis in Appendix K.

## Table 9-1. PRELIMINARY RESULTS OF PILOT STUDY OF BENZENE BODY BURDEN

| Sample | Occupation | Smoker or Non-smoker | Sex | Weight (kg) | Worked or Off Preceding Test | Benzene Levels Found | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Air Monitor | | Breath | | | Blood | Urine |
| | | | | | | ($\mu g/m^3$) | (ppb) | ($\mu g/m^3$) | (ppb) | (ng/min) | μg/ℓ | μg/ℓ |
| A | Laboratory worker | smoker | M | 100 | Worked | a | a | 9.3 | 3.1 | 87 | - | |
| B | Shop worker | smoker | M | 60 | Worked | a | a | 19.0 | 6.5 | 110 | - | |
| C | Filling station attendant | smoker | M | 60-70 | Worked[b]<br>Off | 153<br>- | 48<br>- | 432.0<br>6.8 | 136.0<br>2.1 | 2300<br>60 | 18.6 ± 1.4<br>0.42 ± 0.07 | |
| D | Filling station attendant | smoker | M | 80-90 | Worked<br>Off | 260<br>13 | 82<br>4 | 17.0<br>11.0 | 5.4<br>3.5 | 98<br>57 | 1.88<br><0.35 | |
| E | Filling station attendant | non-smoker | M | 90-100 | Worked<br>Off | -[c]<br>13 | -[c]<br>4 | 7.2<br>1.3 | 2.3<br>0.4 | 40<br>8.4 | 0.28 ± 0.06<br><0.34 | |
| F | Laboratory worker | non-smoker | M | 60 | Worked | a | a | 1.1 | 0.4 | 7.3 | 0.1 | |
| G | Laboratory worker | non-smoker | M | 70-80 | Worked | a | a | 3.7 | 1.2 | 10.4 | d | 0.72 ± 0.14[e] |
| H | Filling station attendant | smoker | M | 60-70 | Worked<br>Off | 190<br>1.4 | 60<br>0.4 | 27<br>15 | 8.4<br>4.7 | 200<br>130 | 1.14<br>0.40 ± 0.06 | 0.93 ± 0.14<br>0.41 ± 0 |
| I | Gasoline tanker driver | non-smoker | M | 60-70 | Worked<br>Off | 54<br>85 | 17<br>27 | 58<br>28 | 18<br>8.4 | 513<br>246 | 1.30 ± 0.34<br>1.93 ± 0.12 | 2.4 ± 0.4<br>1.6 ± 0 |

[a] The individuals were not monitored; however, air monitoring in the vicinity of their work areas indicated 8.2 $\mu g/m^3$ of benzene in the air.

[b] Subject C had been repairing a fuel line on an automobile. It is probable that he was exposed to more benzene than indicated by the personnel monitor.

[c] Cartridge lost due to breakage.

[d] Interfering peak on the gas chromatogram.

[e] Sample taken from the same subject at a later date.

Breath levels of benzene were consistently higher on "working" days than on "off" days. Smokers had consistently higher benzene levels in their breath than non-smokers.

A similar pilot study was performed on a group of nine residents of the "Love Canal" to confirm the extension of this technique to halogenated organics. Here halogenated organics were measured (Table 9-2) as well as benzene (Table 9-3). The benzene levels reflected the individuals smoking habits as in the earlier study. Eighteen halogenated organics were found in breath samples and at least some of the compounds appear to be site-specific (i.e. chlorobenzotrifluoride, dichlorotoluene, hexachlorobutadiene, trichlorobenzene, pentachlorobenzene, chlorotoluene).

## Table 9-2 . ESTIMATED LEVELS OF HALOGENATED COMPOUNDS IN HUMAN BREATH FROM "OLD LOVE" CANAL IN NIAGARA FALLS, NY[a]

| | Participant No. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Halogenated Compounds** | 10009 | 10017 | 10025 | 10033 | 10066 | 10041 | 10058 | 10074 | 10090 |
| 1,2-dichloroethane | -[b] | T | - | 243 ± 108 | - | 136 ± 75 | - | - | 73 ± 12 |
| carbon tetrachloride | 90 ± 36 | 796 ± 135 | 622 | 689 ± 378 | - | 167 ± 75 | T | T | 88 ± 22 |
| trichloroethylene | - | T | - | - | - | T | T | T | - |
| 1,1,1-trichloroethane | 108 ± 36 | 286 ± 122 | 351 | 329 ± 106 | 2,812 | 394 ± 121 | T | T | 264 ± 44 |
| tetrachloroethylene | 658 ± 63 | 1,224 ± 285 | 1,904 | 1,152 ± 129 | 750 | 667 ± 75 | 4,469 | 1,753 | 632 ± 294 |
| 1,1-dichloroethane | - | T | - | - | - | - | - | - | - |
| dichlorobenzene isomer(s)[c] | 459 ± 72 | 755 ± 204 | 513 | 5,294 | T | - | - | T | 58 ± 0 |
| chlorobenzene | - | T | - | - | - | - | - | - | - |
| chloroform | 36,576 | 94,510 | 45,892 | 37,447 | 3,896 | 23,530 | 25,591 | 20,424 | 23,529 ± 11,764 |
| 1,3-hexachlorobutadiene | T | - | - | - | - | - | - | - | - |
| trichlorobenzene isomer(s) | T | - | - | - | - | - | - | - | 88 |
| tetrachlorobenzene isomer(s) | 180 | - | 27 | - | - | - | - | - | - |
| pentachlorobenzene isomer(s) | - | - | - | - | - | - | - | - | 73 |
| chlorotoluene isomer(s) | 261 ± 45 | - | 338 | - | - | - | - | - | - |
| dichlorotoluene isomer(s) | - | - | - | - | - | - | - | - | 1,220 |
| bromotoluene isomer(s) | - | - | - | - | - | - | - | - | T |
| chloronaphthalene isomer(s) | - | - | - | - | - | - | - | - | T |
| chlorobenzotrifluoride isomer(s) | - | T | T | - | - | - | T | T | 852 ± 441 |

[a] Values are in $ng/m^3$, duplicate values were obtained where indicated.

[b] - equals not detected.

[c] Values are summed for all isomers when more than one observed.

Table 9-3 . ESTIMATED LEVELS OF BENZENE IN HUMAN BREATH FROM "OLD LOVE" CANAL IN NIAGARA FALLS, NY

| Participant No. | Benzene ($ng/m^3$) | Comment |
|---|---|---|
| 10009 | 1,837 ± 117 | smoker |
| 10017 | 2,571 ± 1,551 | smoker |
| 10025 | 905 | |
| 10033 | 6,776 ± 776 | smoker |
| 10066 | 687 | |
| 10041 | 6,954 ± 1,651 | smoker |
| 10058 | 898 | |
| 10074 | 4,877 | smoker |
| 10090 | 735 ± 132 | |

# SECTION 10
# FIELD OPERATIONS

Field operations activities have been centered in 4 areas:

1. Establishing communications regarding study activities with appropriate regional, state and local agencies;
2. Developing appropriate instrumentation;
3. Conducting a pretest of data collection instruments and procedures in Niagara Falls, New York; and
4. Preparing the submission for Office of Management and Budget (OMB) review.

More details on these activities is presented in the following sections.

## CONTACT WITH REGIONAL, STATE AND LOCAL AGENCIES

The RTI Project Survey Director has met with representatives of the following agencies to discuss study activities:

Office of Toxic Substances, EPA Region II, Edison, New Jersey
New York State Health Department, Albany, New York
New Jersey State Health Department, Trenton, New Jersey
Niagara County Health Department, Niagara Falls, New York
EPA Region VI, Dallas, Texas
Division of Epidemiology, Texas Department of Health, Austin, Texas
Texas Air Control Board, Austin and Bellaire, Texas
EPA Laboratory, Houston, Texas
Harris County Health Department, Houston, Texas
Harris County Pollution Control Department, Pasadena, Texas
City of Houston Health Department, Houston, Texas
Department of Public Health, Pasadena, Texas
Louisiana Department of Health and Human Resources, New Orleans, LA

The purpose of these meetings was to discuss the study protocol. State and local officials were solicited regarding support for the study, particularly an appropriate individual's name and telephone number to appear on the participant consent form (PCF) should participants have questions or wish to verify the study. Publicity was also discussed, as well as the possibility of certain forms of assistance at the local level, specifically a van to serve as a central data collection facility in sampling areas remote from public buildings and an experienced phlebotomist to assist in the collection of blood samples.

All individuals contacted indicated interest and support for, the study. The general consensus of local officials in Texas was that the study should be low-key without widespread publicity. The Texas Department of Health indicates that they might be of some assistance in securing tissue samples when and if that time comes. They noted that Texas may present some special problems with regard to mortality records and the definition of death. EPA Region II has indicated that a mobile laboratory van will be at RTI's disposal *in lieu* of renting a Winnebago for the main study activites in Region II, if RTI so desires; a similar vehicle may be available from the EPA Laboratory in Houston. Such arrangements would improve EPA's visibility and reduce RTI's field costs.

## PRETEST

In July 1978, a limited pretest of data collection instruments and procedures was executed in the Love Canal area of Niagara Falls, New York, with the cooperation and assistance of EPA Region II, the New York State Health Department, and the Niagara County Health Department. Potential participants were selected from two sources: (1) a list of individuals who had indicated to the New York State Health Department, during that agency's health status screening in the Love Canal area in June 1978, that they would be interested in participating in further study; and (2) a list of households whose basements were sampled by RTI early in 1978. Nine participants were purposely selected so that (1) both residential blocks of the Love Canal area were represented, and (2) the pretest stayed within limits imposed by OMB. Two participants resided in the northernmost block and 7 in the southernmost block, and 2 participants resided in the same household (but were studied over different time periods - see below). In addition, 7 participants were female, 2 male; all were adults. Six participants were selected from the State list, 2 from the list of households monitored earlier by RTI, and 1 participant appeared on both lists. There were no refusals; one participant did not recall telling the State that she would participate in further studies, but she participated anyway. RTI had originally planned to offer a $15 incentive for participation; however, due to the close temporal relationship of the RTI pretest to the State study, which offered no incentive, RTI did not offer an incentive either, on the recommendation of EPA Region II.

EPA Region II arranged with the Niagara Falls school system for RTI to use space at the school in the Love Canal area as a central data collection facility; RTI was granted access to the health room at the school, ideally suited for use as a central data collection facility, replete with refrigerator and sink. The Niagara County Health Department provided the services of one experienced R.N. phlebotomist to assist with the collection of blood samples.

For each participant, a questionnaire (see below) was administered; air monitoring devices were set up inside the house and in the yard; two 120 ml tap water samples were collected before and after flushing the lines for 3 minutes; and arrangements were made for them to come to the school at a specified time where a breath sample was obtained and three 10 ml Vacutainer tubes (two containing sodium heparin as an anticoagulant, one containing liquid EDTA) were filled by brachial venipuncture. At the time of initial contact, each participant was provided with a 120 ml bottle and asked to collect an early morning urine sample, which all did. The indoor and outdoor air monitoring was terminated shortly after the collection of the blood and breath samples. Specific gravity determinations were performed on all urine samples using a protometer.

The first 5 participants were interviewed early on the morning of July 6, and were processed at the central data collection facility that afternoon. The other 4 participants were interviewed that afternoon and processed at the central data collection facility on the morning of July 7. Spot urine samples were collected from participants 1-3 and 5 the afternoon of July 6 at the time the breath and blood samples were collected; however, no attempt was made to collect a spot urine sample from participants 6-9 due to the short time elapsed between the early morning sample and their appointments at the school. For the 2 participants who resided in the same household, only one set of tap water samples was collected at that residence.

Following the pretest, several discussions took place among RTI project principals and between the RTI Project Survey Director and the EPA/OTS Project Officer regarding the release and dissemination of data. Summary data which did not identify individual participants were provided to EPA/OTS, and through them to EPA Region II and the New York State Health Department.

There was concern that direct release of data to participants could result in gross misinterpretation and unnecessary anxiety. Therefore, the decision was made to provide the participant-data linkage to the New York State Health Department only for appropriate action. That agency had consent/release forms from virtually every resident of the Love Canal area which had been the basis of their providing RTI with the list of names which yielded 7 of the 9 participants in the RTI pretest. RTI obtained copies of those consent forms for all 9 participants, then discussed both consent forms, New York's and RTI's (the RTI consent form specifically mentions cooperation with the New York State Health Department - see below), with the Director of RTI's Survey Operations Center and Mr. Kenneth R. Wing, Assistant Professor of Health Law at the University of North Carolina and an RTI consultant. These individuals saw no problems with the RTI-New York State Health Department interaction.

## INSTRUMENTATION

Appendix N presents a copy of the PCF and Questionnaire used in the pretest. Following the pretest, these instruments were revised and in March 1979 were submitted to EPA for review and submission to OMB. The final version of the Household Screening Questionnaire, the PCF, and the Questionnaire appear in Appendix O.

# APPENDIX A

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN AMBIENT AIR

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN AMBIENT AIR

## 1.0 Principle of Method

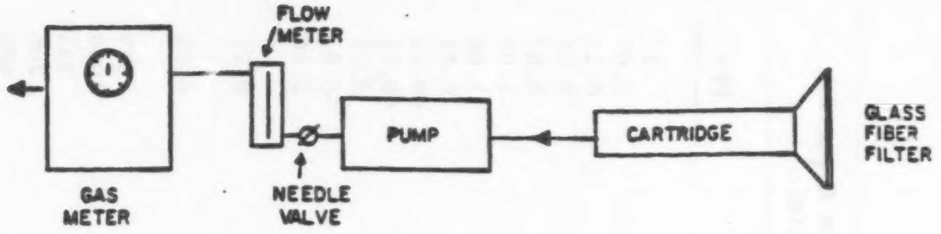
Volatile organic compounds are concentrated from ambient air onto Tenax GC in a short glass tube (A1-A3). Recovery of the volatile organics is accomplished by thermal desorption and purging with helium into a liquid nitrogen cooled nickel capillary trap (A1-A2,A4) and then the vapors are introduced into a high resolution glass gas chromatographic column where the constituents are separated from each other (A2,A5). Characterization and quantification of the constituents in the sample are accomplished by mass spectrometry either by measuring the intensity of the total ion current signal or by mass fragmentography (A2,A6). The collection and analysis systems are shown in Figure A-1.

## 2.0 Range and Sensitivity

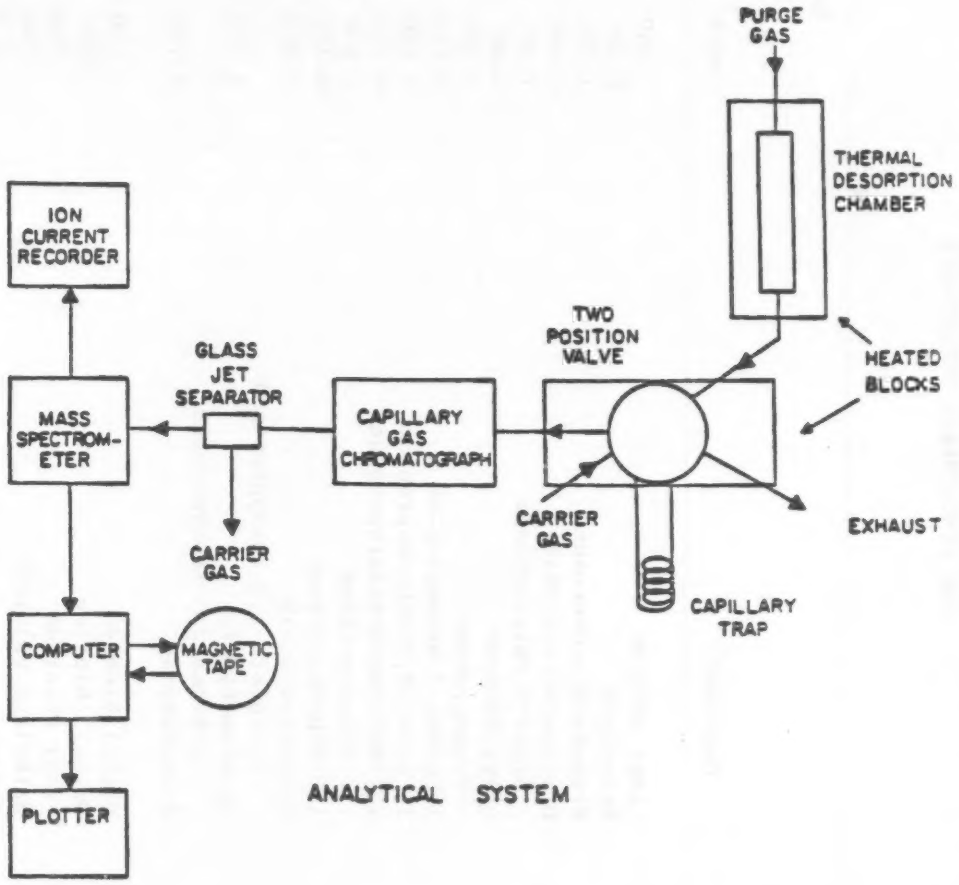
The linear range for the analysis of volatile organic compounds depends upon two principal features. The first is a function of the breakthrough volume of each specific compound which is trapped on the Tenax GC sampling cartridge and the second is related to the inherent sensitivity of the mass spectrometer for each organic (A2,A7). Thus, the range and sensitivity are a direct function of each compound which is present in the original ambient air. The linear range for quantitation on the gas chromatograph/mass spectrometer/computer (GC/MS/COMP) is generally three orders of magnitude. Table A-1 lists the overall theoretical sensitivity for some examples of volatile organics which is based on these two principles (A7).

## 3.0 Interferences

The potential difficulties with this technique are primarily associated with those cases where isomeric forms of a particular substance cannot be resolved by the high resolution chromatographic column and when the mass spectral fragmentation patterns of each of the isomers are identical. An example of such a problem is seen with the $C_5$-alkyl aromatics of which there are 53 isomers. As the number of carbon atoms increases in the hydrocarbons and aromatics, the number of potential isomers becomes increasingly large


FLOW METER
GAS METER
NEEDLE VALVE
PUMP
CARTRIDGE
GLASS FIBER FILTER

VAPOR COLLECTION SYSTEM


PURGE GAS
THERMAL DESORPTION CHAMBER
ION CURRENT RECORDER
TWO POSITION VALVE
HEATED BLOCKS
GLASS JET SEPARATOR
MASS SPECTROM-ETER
CAPILLARY GAS CHROMATOGRAPH
CARRIER GAS
EXHAUST
CARRIER GAS
CAPILLARY TRAP
COMPUTER
MAGNETIC TAPE
PLOTTER

ANALYTICAL SYSTEM

Figure A-1. Vapor collection and analytical systems for analysis of organic vapors in ambient air.

## Table A-1. OVERALL THEORETICAL SENSITIVITY OF HIGH RESOLUTION GAS CHROMATOGRAPHY/MASS SPECTROMETRY/COMPUTER ANALYSIS FOR ATMOSPHERIC POLLUTANTS

| Chemical Class | Compound | Estimated Detection Limit[a] | |
|---|---|---|---|
| | | $ng/m^3$ | ppt |
| Halogenated hydrocarbon | Vinyl bromide | 250 | 57 |
| | Bromoform | 0.340 | 0.03 |
| | Bromodichloromethane | 1.300 | 0.22 |
| | Dibromochloromethane | 0.667 | 0.07 |
| | 1-Bromo-2-chloroethane | 1.00 | 0.67 |
| | Allyl bromide | 5.00 | 1.04 |
| | 1-Bromopropane | 5.200 | 1.06 |
| | 1-Chloro-3-bromopropane | 0.150 | 0.01 |
| | 1-Chloro-2,3-dibromopropane | ~0.100 | <0.01 |
| | 1,1-Dibromo-2-chloropropane | ~0.100 | <0.01 |
| | 1,2-Dibromoethane | 0.530 | 0.07 |
| | 1,3-Dibromopropane | ~0.100 | ~0.01 |
| | Epichlorohydrin (1-Chloro-2,3-epoxypropane) | 9.600 | 2.50 |
| | Epibromohydrin (1-Bromo-2,3-epoxypropane) | 0.300 | 0.05 |
| | Bromobenzene | 0.100 | 0.02 |
| | Methyl bromide | 500 | 135 |
| | Methyl chloride | 2000 | 1000 |
| | Vinyl chloride | 800 | 333 |
| | Methylene chloride | 700 | 200 |
| | Chloroform | 200 | 420 |
| | Carbon tetrachloride | 250 | 400 |

(continued)

Table A-1 (cont'd.)

| Chemical Class | Compound | Estimated Detection Limit[a] ng/$m^3$ | Estimated Detection Limit[a] ppt |
|---|---|---|---|
| Halogenated hydrocarbon (cont'd) | 1,2-Dichloroethane | 32 | 8.15 |
| | 1,1,1-Trichloroethane | 66 | 12.45 |
| | Tetrachloroethylene | 2.5 | 0.38 |
| | Trichloroethylene | 10 | 1.92 |
| | 1-Chloro-2-methylpropene | 62 | 21.5 |
| | 3-Chloro-2-methylpropene | 62 | 21.5 |
| | 3-Chloro-1-butene | 83 | 28.8 |
| | Allyl chloride | 83 | 28.8 |
| | 4-Chloro-1-butene | 38 | 13.2 |
| | 1-Chloro-2-butene | 13 | 4.5 |
| | Chlorobenzene | 2.10 | 0.47 |
| | o-Dichlorobenzene | 1.00 | 0.06 |
| | m-Dichlorobenzene | 0.75 | 0.01 |
| | Benzylchloride | 0.65 | 0.01 |
| Halogenated ethers | 2-Chloroethyl ethyl ether | 4.15 | 0.97 |
| | Bis-(chloromethyl)ether | 1.0 | 1.10 |
| Nitrosamines | N-Nitrosodimethylamine | 5.0 | 1.67 |
| | N-Nitrosodiethylamine | 3.0 | 0.74 |
| Oxygenated hydrocarbons | Acrolein | ~100 | 56.5 |
| | Glycidaldehyde | ~59 | 9.5 |
| | Propylene oxide | ~60 | 25.5 |

(continued)

Table A-1 (cont'd.)

| Chemical Class | Compound | Estimated Detection Limit[a] | |
|---|---|---|---|
| | | ng/m$^3$ | ppt |
| Oxygenated hydrocarbons (cont'd) | Butadiene diepoxide | ~20 | 6.7 |
| | Cyclohexene oxide | ~10 | 2.5 |
| | Styrene oxide | 2 | 0.415 |
| | Acetophenone | ~2 | ~0.415 |
| | β-Propiolactone | ~3 | ~1.2 |
| Nitrogenous Compounds | Nitromethane | 8 | ~2.4 |
| | Aniline | 3.0 | 0.78 |
| Sulfur Compounds | Diethyl sulfate | ~50 | - |
| | Ethyl methane sulfate | ~5.0 | - |

[a] Limits are calculated on the basis of the breakthrough volume for 2.2 g of Tenax GC, (at 70°F), capillary column performance and sensitivity of the mass spectrometer to that compound in the mass fragmentography mode of most intense ion.

and difficult to completely resolve by gas chromatography and/or by their corresponding mass spectral fragmentation patterns. However, differentiation between the hydrocarbons, that is, alkanes, alkenes, aromatics, oxygenated, etc., can be accomplished.

## 4.0 Reproducibility

The reproducibility of this method has been determined to range from ±10 to ±30% for different substances when replicate sampling cartridges are examined (A5). The inherent analytical errors are a function of several factors: [1] the ability to accurately determine the breakthrough volume for each of the organic compounds identified; [2] the accurate measurement of the ambient air volume sampled; [3] the percent recovery of the organic from the sampling cartridge after a period of storage; [4] the reproducibility of thermal desorption for a compound from the cartridge and its introduction into the analytical system; [5] the accuracy of determining the relative molar response ratios between the identified substance and the external standard used for calibrating the analytical system; [6] the reproducibility of transmitting the sample through the high resolution gas chromatographic column; and [7] the day-to-day reliability of the MS/COMP system (A1-A8).

The accuracy of analysis is generally ±30% but depends on the chemical and physical nature of the compound (A2,A8).

## 5.0 Advantages and Disadvantages of the Method

The gas chromatograph/mass spectrometer interfaced with a jet separator is extremely sensitive and specific for the analysis of many volatile organic compounds in ambient air. High resolution gas chromatographic separation provides adequate resolution of the substances found in ambient air for their subsequent quantification. The combination of the high resolution gas chromatographic column and the selection of specific or unique ions representing the various compounds of interest identified in the air samples yields a relatively specific assay method for these compounds (A1-A8).

Collected samples can be stored up to one month with less than 10% losses for most of the chemical classes (A2,A8). Because some of the compounds of interest may be hazardous to man, it is extremely important to exercise safety precautions in the preparation and disposal of liquid and gas standards, cleaning of used glassware, etc. in the analysis of air samples.

The efficiency of air sampling increases as the ambient air temperature decreases (i.e., sensitivity increases) (A8).

The retention of water by Tenax is low; its thermal stability is high and its background is negligible, allowing sensitive analysis (A1,A2,A5,A8).

## 6.0 Apparatus

### 6.1 Sampling Cartridges

The sampling tubes are prepared by packing a ten centimeter long by 1.5 cm i.d. glass tube containing 6 cm of 35/60 mesh Tenax GC with glass wool in the ends to provide support (A2,A5). Tenax is extracted in a Soxhlet apparatus for a minimum of 24 hours each time with methanol and pentane prior to preparation of cartridge samplers (A2,A5). After purification of the Tenax GC sorbent and drying in a vacuum oven at 100°C for 3 to 5 hours at 28 inches of water. All the sorbent material is meshed to provide a 35/60 particle size range. Cartridge samplers are then prepared and conditioned at 270°C with helium flow at 30 ml/min for 30 min. The conditioned cartridges are transferred to Kimax® (2.5 cm x 150 cm) culture tubes, immediately sealed using Teflon-lined caps and cooled. This procedure is performed in order to avoid recontamination of the sorbent bed (A2,A5).

Cartridge samplers with longer beds of sorbent may be prepared using a proportionally increased amount of Tenax in order to achieve a larger breakthrough volume for compounds of interest, and thus increasing the overall sensitivity of the technique (A8).

### 6.2 Gas Chromatographic Column

A 0.35 mm i.d. x 100 m glass SCOT capillary column coated with SE-30 stationary phase is used for effecting the resolution of the volatile organic compounds (A5). The capillary column is conditioned for 48 hrs. at 245° at 2.25 ml/min of helium flow.

A glass jet separator is employed to interface the glass capillary column to the mass spectrometer on the Varian MAT CH-7 GC/MS/COMP system and on the Finnigan 3300 system. The glass jet separators are maintained at 240°C (A2,A5). A metal jet separator, maintained at 210°C, is employed to interface the LKB 2091 GC/MS/COMP system to the glass capillary column.

## 6.3 Inlet Manifold

An inlet manifold for thermally recovering vapors trapped on Tenax sampling cartridges is used and is shown in Figure A-1 (A1,A2,A4,A5).

## 6.4 Gas Chromatograph

A Varian 1700 gas chromatograph is used to house the glass capillary column and is interfaced to the inlet manifold on the Varian MAT CH-7 system. A Finnigan 9500 gas chromatograph houses the glass capillary column and is interfaced to the thermal desorption inlet manifold on the Finnigan 3300 system. A Pye-Unicam 104 gas chromatograph is used to house the glass capillary column and is interfaced to the thermal desorption inlet manifold on the LKB 2091 system. These analytical systems are presented schematically in Figure A-1.

## 6.5 Mass Spectrometer/Computer

A Varian MAT CH-7 mass spectrometer capable of a resolution of 2,000 equipped with single ion monitoring capability is used in tandem with the Varian 1700 gas chromatograph and interfaced to a Varian 620/L computer (Figure A-1). A Finnigan 3300 mass spectrometer (unit resolution over a mass range of 1,000 daltons) equipped with selected ion monitoring capability (nine channels available) is used in tandem with the Finnigan 9500 gas chromatograph and interfaced to a PDP-12 computer (Figure A-1). An LKB 2091 mass spectrometer capable of a resolution of 1,000 equipped with selected ion monitoring capability (sixteen channels available) is used in tandem with the Pye-Unicam 104 gas chromatograph and interfaced to a PDP-11/04 computer (Figure A-1).

## 7.0 Reagents and Materials

All reagents used are analytical reagent grade.

## 8.0 Procedure

## 8.1 Cleaning of Glassware

All glassware, sampling tubes, cartridge holders, etc. are washed in Isoclean/water, rinsed with deionized distilled water, acetone and air dried. Glassware is heated to 450-500°C for 2 hours to insure that all organic material has been removed prior to its use.

## 8.2 Collection of Volatile Organics in Ambient Air

Continuous sampling of ambient air is accomplished using a Nutech Model 221-A portable sampler (Nutech Corp., Durham, NC, see Figure A-1, Reference 2). Flow rates between 1-10 l/min are available with this sampling system. Flow rates are generally maintained at 1 l using critical orifices and the total flow is monitored through a calibrated flow meter. The total flow is also registered by a dry gas meter. This portable sampling unit operates on a 12 volt storage battery and is capable of continuous operation up to a period of 24 hours. However, in most cases, at the rates which are employed in the field, the sampling period is generally 1-3 hours. This portable sampling unit is generally utilized for obtaining "high volume" samples. Duplicate cartridges are deployed on each sampling unit utilizing a sampling head as shown in Figure A-2.

Concomitant with these parameters the temperature is continuously recorded with a Meterological Research, Inc. Weather Station since the breakthrough volume is important in order to obtain quantitative data on the volatile organics.

In addition to the Nutech samplers, personnel samplers (DuPont and MSA) are also used to acquire "low volumes" of ambient air as well as long-term integrated samples (12-36 hrs.) Identical Tenax GC sampling cartridges are employed in this case, and the sampling is conducted in duplicate. The flow rate is balanced between duplicate cartridges using critical orifices to maintain a rate of 25-100 ml/min per cartridge.

For large sample volumes, it is important to realize that the total volume of air may cause the elution of compounds through the sampling tube if their breakthrough volume is exceeded. The breakthrough volumes of some of the volatile organics are shown in Table A-2 (A2,A4,A8). These breakthrough volumes have been determined by a previously described technique (A2). The breakthrough volume is defined as that point at which 50% of a discrete sample introduced into the cartridge is lost. For cases in which the identity of a volatile organic compound is not known until after GC/MS analysis, the breakthrough volume may be subsequently determined.

Previous experiments have shown that the organic vapors collected on Tenax GC sorbent are stable and can be quantitatively recovered from the

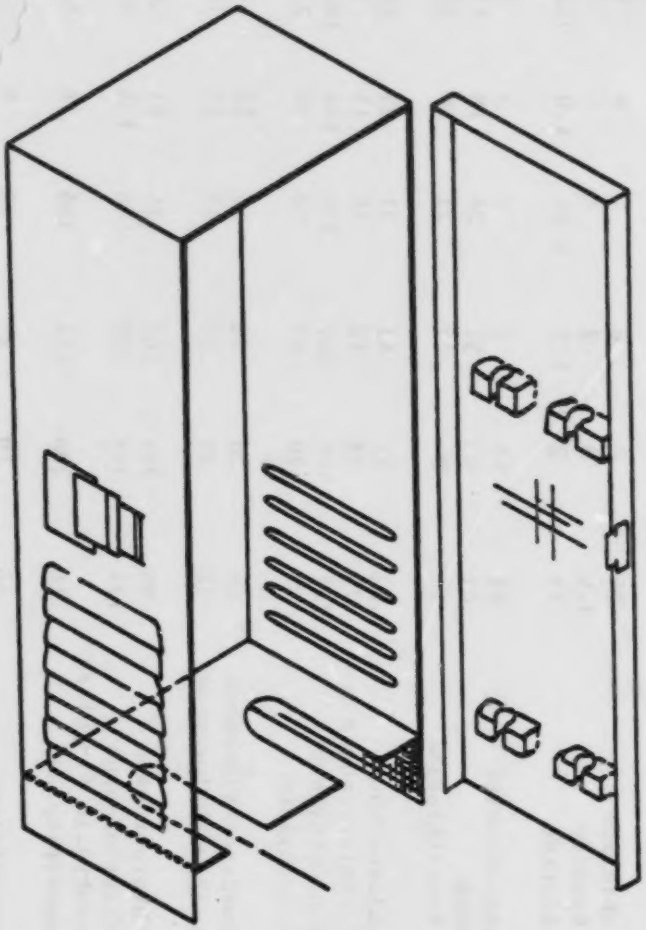


Figure A-2. Sampling head for housing cartridge sampling train.

Table A-2. TENAX GC BREAKTHROUGH VOLUMES FOR SEVERAL ATMOSPHERIC POLLUTANTS[1]

| Chemical Class | Compound | b.p. (°C) | Temperature (°F) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 50 | 60 | 70 | 80 | 90 | 100 |
| Halogenated hydrocarbon | methyl chloride | -24 | 8 | 6 | 5 | 4 | 3 | 2.5 |
| | methyl bromide | 3.5 | 3 | 2 | 2 | 1 | 1 | 0.9 |
| | vinyl chloride | 13 | 2 | 1.5 | 1.25 | 1.0 | 0.8 | 0.6 |
| | methylene chloride | 41 | 11 | 9 | 7 | 5 | 4 | 3 |
| | chloroform | 61 | 42 | 31 | 24 | 18 | 13 | 10 |
| | carbon tetrachloride | 77 | 34 | 27 | 21 | 16 | 13 | 10 |
| | 1,2-dichloroethane | 83 | 53 | 41 | 31 | 23 | 18 | 14 |
| | 1,1,1-trichloroethane | 75 | 23 | 18 | 15 | 12 | 9 | 7 |
| | tetrachloroethylene | 121 | 361 | 267 | 196 | 144 | 106 | 78 |
| | trichloroethylene | 87 | 90 | 67 | 50 | 38 | 28 | 21 |
| | 1-chloro-2-methylpropene | 68 | 26 | 20 | 16 | 12 | 9 | 7 |
| | 3-chloro-2-methylpropene | 72 | 29 | 22 | 17 | 13 | 10 | 8 |
| | 1,2-dichloropropane | 95 | 229 | 162 | 115 | 81 | 58 | 41 |
| | 1,3-dichloropropane | 121 | 348 | 253 | 184 | 134 | 97 | 70 |
| | epichlorohydrin (1-chloro-2,3-epoxypropane) | 116 | 200 | 144 | 104 | 74 | 54 | 39 |
| | 3-chloro-1-butene | 64 | 19 | 15 | 12 | 9 | 7 | 6 |
| | allyl chloride | 45 | 21 | 16 | 12 | 9 | 6 | 5 |
| | 4-chloro-1-butene | 75 | 47 | 36 | 27 | [illegible]0 | 15 | 12 |
| | 1-chloro-2-butene | 84 | 146 | 106 | 77 | 56 | 40 | 29 |
| | chlorobenzene | 132 | 899 | 653 | 473 | 344 | 249 | 181 |
| | o-dichlorobenzene | 181 | 1,531 | 1,153 | 867 | 656 | 494 | 372 |
| | m-dichlorobenzene | 173 | 2,393 | 1,758 | 1,291 | 948 | 697 | 510 |

(continued)

Table A-2 (cont'd.)

| Chemical Class | Compound | b.p. (°C) | Temperature (°F) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 50 | 60 | 70 | 80 | 90 | 100 |
| Halogenated hydrocarbons (cont'd) | benzyl chloride | 179 | 2,792 | 2,061 | 1,520 | 1,125 | 830 | 612 |
| | bromoform | 149 | 507 | 386 | 294 | 224 | 171 | 131 |
| | ethylene dibromide | 131 | 348 | 255 | 188 | 138 | 101 | 74 |
| | bromobenzene | 155 | 2,144 | 1,521 | 1,079 | 764 | 542 | 384 |
| Halogenated Ethers | 2-chloroethyl ethyl ether | 108 | 468 | 336 | 241 | 234 | 124 | 89 |
| | Bis-(chloromethyl)ether | - | 995 | 674 | 456 | 309 | 209 | 142 |
| Nitrosamines | N-nitrosodimethylamine | 151 | 385 | 280 | 204 | 163 | 148 | 107 |
| | N-nitrosodiethylamine | 177 | 2,529 | 1,836 | 1,330 | 966 | 700 | 508 |
| Oxygenated hydrocarbons | acrolein | 53 | 19 | 14 | 10 | 8 | 6 | 4 |
| | glycidaldehyde | - | 364 | 247 | 168 | 114 | 77 | 52 |
| | propylene oxide | 34 | 35 | 24 | 17 | 11 | 8 | 5 |
| | butadiene diepoxide | - | 1,426 | 1,009 | 714 | 506 | 358 | 253 |
| | cyclohexene oxide | 132 | 2,339 | 1,644 | 1,153 | 811 | 570 | 400 |
| | styrene oxide | 194 | 5,370 | 3,926 | 2,870 | 2,094 | 1,531 | 1,119 |
| | phenol | 183 | 2,071 | 1,490 | 1,072 | 769 | 554 | 398 |
| | acetopheonone | 202 | 3,191 | 2,382 | 1,778 | 1,327 | 991 | 740 |
| | β-propiolactone | 57 | 721 | 514 | 366 | 261 | 186 | 132 |
| Nitrogenous Hydrocarbons | nitromethane | 101 | 45 | 34 | 25 | 19 | 14 | 11 |
| | aniline | 184 | 3,864 | 2,831 | 2,075 | 1,520 | 1,114 | 817 |
| Sulfur Compounds | diethyl sulfate | 208 | 40 | 29 | 21 | 15 | 11 | 8 |
| | ethyl methane sulfate | 86 | 5,093 | 3,681 | 2,564 | 1,914 | 1,384 | 998 |

(continued)

Table A-2 (cont'd.)

| Chemical Class | Compound | b.p. (°C) | Temperature (°F) 50 | 60 | 70 | 80 | 90 | 100 |
|---|---|---|---|---|---|---|---|---|
| Amines | dimethylamine | 7.4 | 9 | 6 | 4 | 3 | 2 | 1 |
| | isobutylamine | 69 | 71 | 47 | 34 | 23 | 16 | 11 |
| | *t*-butylamine | 89 | 6 | 5 | 4 | 3 | 2 | 1 |
| | di-(*n*-butyl)amine | 159 | 9,506 | 7,096 | 4,775 | 3,105 | 2,168 | 1,462 |
| | pyridine | 115 | 378 | 267 | 189 | 134 | 95 | 67 |
| | aniline | 184 | 8,128 | 5,559 | 3,793 | 2,588 | 1,766 | 1,205 |
| Ethers | diethyl ether | 34.6 | 29 | 21 | 15 | 11 | 8 | 5 |
| | propylene oxide | 35 | 13 | 9 | 7 | 5 | 4 | 3 |
| Esters | ethyl acetate | 77 | 162 | 108 | 72 | 48 | 32 | 22 |
| | methyl acrylate | 80 | 164 | 111 | 75 | 50 | 34 | 23 |
| | methyl methacrylate | 100 | 736 | 484 | 318 | 209 | 137 | 90 |
| Ketones | acetone | 56 | 25 | 17 | 12 | 8 | 6 | 4 |
| | methyl ethyl ketone | 80-2 | 82 | 57 | 39 | 27 | 19 | 13 |
| | methyl vinyl ketone | 81 | 84 | 58 | 40 | 28 | 19 | 14 |
| | acetophenone | 202 | 5,346 | 3,855 | 2,767 | 2,000 | 1,439 | 1,037 |
| Aldehydes | acetaldehyde | 20 | 3 | 2 | 2 | 1 | 0.9 | 0.7 |
| | benzaldehyde | 179 | 7,586 | 5,152 | 3,507 | 2,382 | 1,622 | 1,101 |
| Alcohols | methanol | 64.7 | 1 | 1 | 0.8 | 0.6 | 0.4 | 0.3 |
| | *n*-propanol | 97.4 | 27 | 20 | 14 | 10 | 7 | 5 |
| | allyl alcohol | 97 | 32 | 23 | 16 | 11 | 8 | 6 |

(continued)

Table A-2 (cont'd.)

| Chemical Class | Compound | b.p. (°C) | Temperature (°F) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 50 | 60 | 70 | 80 | 90 | 100 |
| Aromatics | benzene | 80.1 | 108 | 77 | 54 | 38 | 27 | 19 |
| | toluene | 110.6 | 494 | 348 | 245 | 173 | 122 | 86 |
| | ethylbenzene | 136.2 | 1,393 | 984 | 693 | 487 | 344 | 243 |
| | cumene | 152.4 | 3,076 | 2,163 | 1,525 | 1,067 | 750 | 527 |
| Hydrocarbons | n-hexane | 68.7 | 32 | 23 | 17 | 12 | 9 | 6 |
| | n-heptane | 98.4 | 143 | 104 | 75 | 55 | 39 | 29 |
| | 1-hexene | 63.5 | 28 | 20 | 15 | 11 | 8 | 6 |
| | 1-heptene | 93.6 | 286 | 196 | 135 | 93 | 64 | 44 |
| | 2,2-dimethylbutane | 49.7 | 0.5 | 0.4 | 0.3 | 0.2 | 0.2 | 0.1 |
| | 2,4-dimethylpentane | 80.5 | 435 | 252 | 146 | 84 | 49 | 28 |
| | 4-methyl-1-pentene | 53.8 | 14 | 10 | 8 | 6 | 4 | 3 |
| | cyclohexane | 80.7 | 49 | 36 | 26 | 19 | 14 | 10 |
| Inorganic gases | nitric oxide | - | 0 | 0 | 0 | 0 | 0 | 0 |
| | nitrogen dioxide | - | 0 | 0 | 0 | 0 | 0 | 0 |
| | chlorine | - | 0 | 0 | 0 | 0 | 0 | 0 |
| | sulfur dioxide | - | 0.06 | 0.05 | 0.03 | 0.02 | 0.02 | 0.01 |
| | water | 100 | 0.06 | 0.05 | 0.04 | 0.03 | 0.01 | 0 |

[a] Breakthrough volume is given in $\ell$/2.2 g Tenax GC used in sampling cartridges.

cartridge samplers up to 4 weeks after sampling when they are tightly closed in cartridge holders and placed in a second container that can be sealed, protected from light and stored at 0°C (A1,A2).

## 8.3 Analysis of Samples

The instrumental conditions for the analysis of volatile organics on the sorbent Tenax GC sampling cartridge is shown in Table A-3. The thermal desorption chamber and the six port Valco valve are maintained at 270° and 240°, respectively. The helium purge gas through the desorption chamber is adjusted to 15-20 ml/min. The nickel capillary trap on the inlet manifold is cooled with liquid nitrogen. In a typical thermal desorption cycle, a sampling cartridge is placed in the preheated desorption chamber and the helium gas is channeled through the cartridge to purge the vapors into the liquid nitrogen capillary trap [the inert activity of the trap has been shown in a previous study (A5)]. After the desorption has been completed, the six-port valve is rotated and the temperature on the capillary loop is rapidly raised (greater than 100°/min); the carrier gas then introduces the vapors onto the high resolution GC column. The glass capillary column is temperature programmed from ambient to 240°C at 4°C/min and held at the upper limit for a minimum of 10 min. After all the components have eluted, the column is cooled to ambient temperature and the next sample is processed (A2).

An example of the analysis of volatile organics in ambient air is shown in Figure A-3 and the background from a blank cartridge is shown in Figure A-4. The high resolution glass capillary column was coated with SE-30 stationary phase which is capable of resolving a multitude of compounds to allow their subsequent identification by MS/COMP techniques; in this case over 110 compounds were identified in this chromatogram.

### 8.3.1 Operation of the MS/COMP System (Figure A-5)

#### 8.3.1.1 Varian MAT CH-7

Typically the Varian MAT CH-7 mass spectrometer is first set to operate in the repetitive scanning mode. In this mode the magnet is automatically scanned exponentially upward from a preset low mass to a high mass value. Although the scan range may be varied depending on the particular sample, typically the range is set from approximately $m/z$ 20 to $m/z$ 350. The scan

Table A-3. OPERATING PARAMETERS FOR GLC-MS-COMP SYSTEM

| Parameter | Setting |
|---|---|
| Inlet-manifold | |
| desorption chamber and valve | 270°C |
| capillary trap - minimum | -195°C |
| maximum | 240°C |
| thermal desorption time | 8 min |
| He purge flow | 15 ml/min |
| GLC | |
| 100 m glass SCOT SE-30 | 25-240°C, 4°C/min |
| carrier (He) flow | ~3 ml/min |
| MS<br>Varian MAT CH-7 | |
| scan range | m/z 20 → 350 |
| scan cycle, automatic-cyclic | 1 sec/decade |
| filament current | 300 μA |
| multiplier | 4.0 |
| ion source vacuum | $\sim 4 \times 10^{-6}$ torr |
| Finnigan 3300 | |
| scan range | m/z 20 → 350 |
| scan cycle, automatic-cyclic | 2 sec/cycle |
| filament current | 0.50 mA |
| multiplier | 1.7 kV |
| ion source vacuum | $\sim 1 \times 10^{-5}$ torr |
| LKB 2091 | |
| mass range | m/z 5 → 492 |
| scan cycle, automatic-cyclic | 2.4 sec/cycle (parabolic scan) |
| filament current | 4A |
| multiplier | 300 |
| ion source vacuum | $3 \times 10^{-6}$ torr |

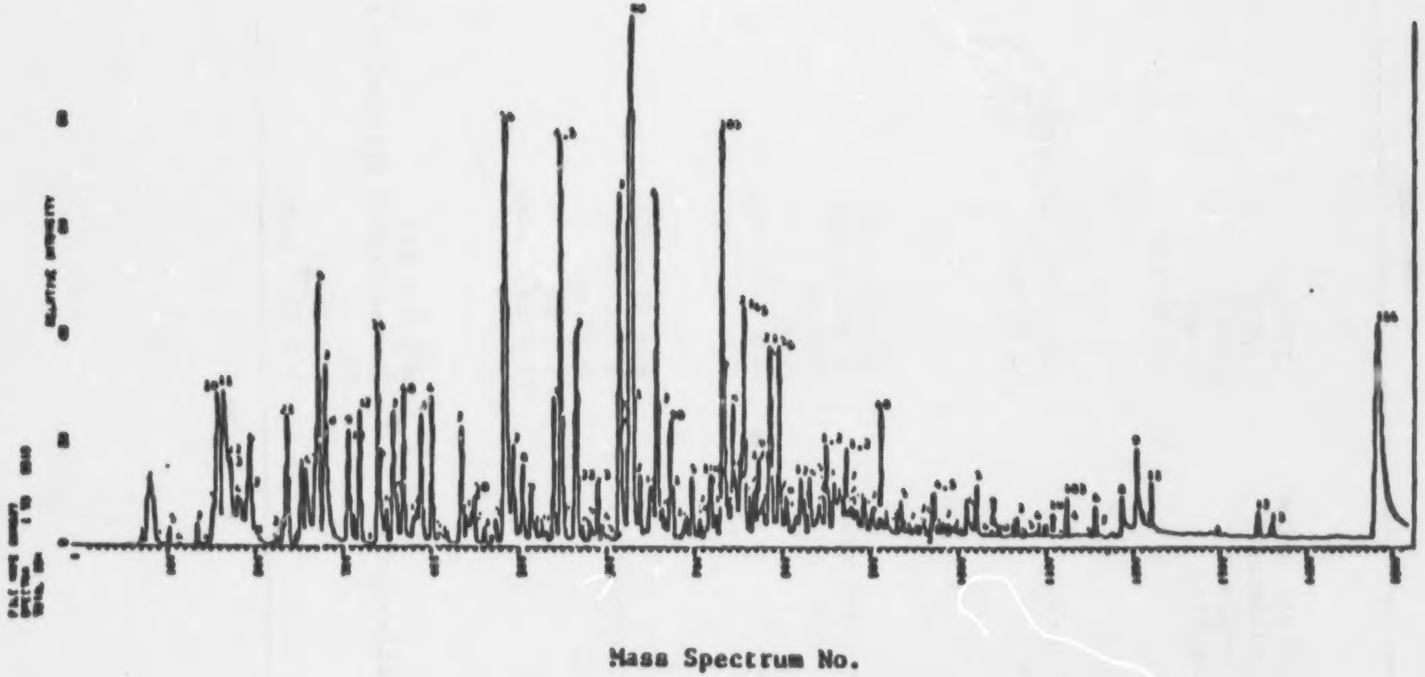


Figure A-3. Profile of ambient air pollutants obtained using high resolution gas chromatography/mass spectrometry/computer.

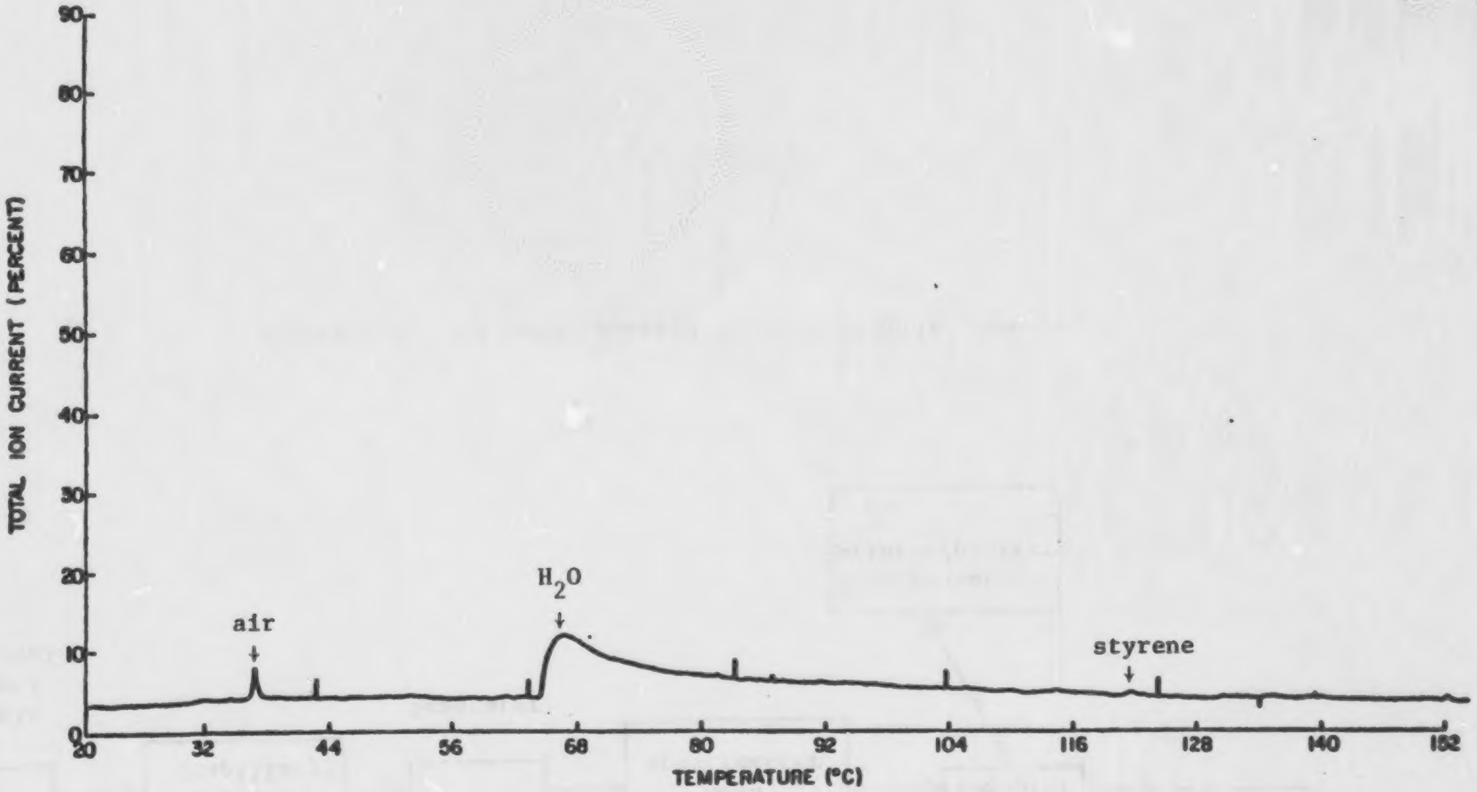


Figure A-4. Background profile for Tenax GC cartridge blank.

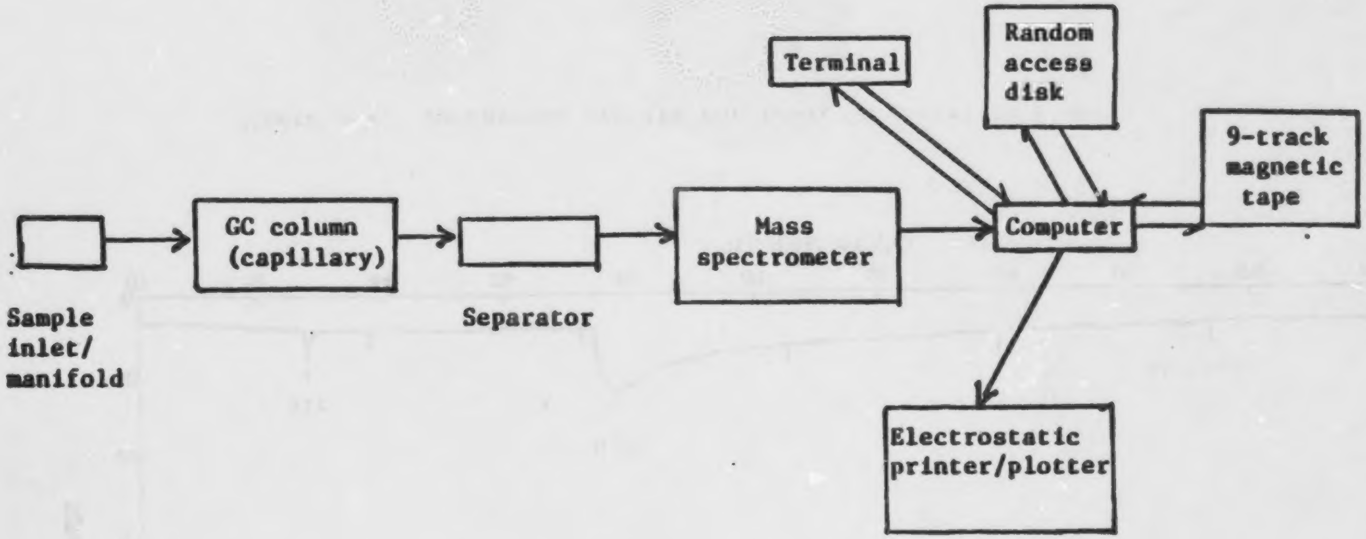

Sample
inlet/
manifold
GC column
(capillary)
Separator
Mass
spectrometer
Terminal
Random
access
disk
Computer
9-track
magnetic
tape
Electrostatic
printer/plotter

Figure A-5. Schematic diagram of GC-MS computer system.

is completed in approximately 1.8 seconds. At this time the instrument automatically resets itself to the low mass position in preparation for the next scan, and the information is accumulated by an on-line 620/L computer and written onto magnetic tapes. The reset period requires approximately 2.0 seconds. Thus, a continuous scan cycle of 3.8 seconds/scan is maintained and repetitively executed throughout the chromatographic run. The result is the accumulation of a continuous series of mass spectra throughout the chromatographic run in sequential fashion.

Prior to analysis of unkrown samples, the system is calibrated by introducing a standard substance, perfluorokerosene, into the instrument and determining the time of appearance of the known standard peaks in relation to the scanning magnetic field. The calibration curve thus generated is stored in the 620/L computer memory. This calibration serves only to calibrate the ions over the scanning range.

While the magnet is continuously scanning, the sample is injected and automatic data acquisition is initiated. As each spectrum is acquired by the computer, each peak which exceeds a preset threshold is recognized and reduced to centroid time and peak intensity. This information is stored in the computer core while the scan is in progress. In addition, approximately 30 total ion current values and an equal number of Hall probe signals are stored in the core of the computer as they are acquired. During the two-second period between scans this spectral information, along with the spectrum number, is written sequentially on magnetic tape, and the computer is reset for the acquisition of the next spectrum.

This procedure continues until the entire GC run is completed. By this time there are from 800-1400 spectra on the magnetic tape which are then subsequently processed. Depending on the information required, they may then either be processed immediately or additional samples may be run, stored on magnetic tape and the results examined at a later time.

The mass spectral data are processed in the following manner. First, the original spectra are scanned and the total ion current (TIC) information is extracted. Then the TIC intensities are plotted against the spectrum number on the plotter. The information will generally indicate whether the run is suitable for further processing, since it provides some idea of the number of unknowns in the sample and the resolution obtained using the particular GC column conditions.

The next stage of the processing involves the conversion of the spectral peak times to peak masses which is done directly via the dual disk system. The mass conversion is accomplished by use of the calibration table obtained previously using perfluorokerosene. Normally one set of the calibration data is sufficient for an entire day's data processing since the characteristics of the Hall probe are such that the variation in calibration is less than 0.2 daltons/day. A typical time required for this conversion process for 1,000 spectra is approximately 30 min.

After the spectra are obtained in mass-converted form, processing proceeds either manually or by computer. In the manual mode, the full spectrum of scans for the GC run is recorded on the Statos 31 plotter. The TIC information available at this time is most useful for deciding which spectra are to be analyzed. At the beginning of the runs where peaks are very sharp nearly every spectrum must be inspected individually to determine the identity of the component. Later in the chromatographic run when the peaks are broader only selected scans need to be analyzed.

#### 8.3.1.2 Finnigan 3300

The Finnigan 3300 mass spectrometer is set to operate in the repetitive scanning mode, scanning the quadrupole from low mass to high mass. A typical range is from $m/z$ 20 to $m/z$ 350. The scan requires approximately one second; the instrument then rests at the high end of the mass scale and then automatically re-initiates a scan cycle at the low mass. During the rest/reset period, data from the previous scan are accumulated by an on-line PDP-12 computer and written on disk. The scan cycle of 2.0 seconds is repetitively executed throughout the chromatographic run, resulting in the accumulation of a continuous series of mass spectra.

The quadrupole mass spectrometer acquires data as masses and intensities. A calibration table is obtained by use of perfluorotributylamine (FC-43) to correlate voltages and masses. Normally one set of calibration data will suffice for a day of data acquisition. The data can then be presented as TIC information (obtained by summing ion intensities over the entire spectrum) with chromatographic peak intensities plotted against time. This information is most useful to decide which spectra should be output and analyzed. At the beginning of the analysis where chromatographic peaks are very sharp nearly every spectrum must be plotted and inspected individually to identify every component. At longer elution times the peaks broaden and only selected scans must be analyzed.

#### 8.3.1.3 LKB 2091

The LKB 2091 mass spectrometer is operated under computer control. The start time and scan interval time are selected by the operator. Typically the magnet is scanned from $\underline{m}/\underline{z}$ 5 to $\underline{m}/\underline{z}$ 490 at a scan speed of 2 sec full scan. The scan cycle time is typically 2.4 secs. Mass vs. intensity data is acquired by the computer and is stored on a disk. The mass values are determined by the LKB mass marker and modified as necessary by a computer program which corrects for deviations due to scan speed. The mass calibration tables are stable for over a six month period of time. The data can then be plotted as total ion current (over an operator determined mass range-usually $\underline{m}/\underline{z}$ 33 → $\underline{m}/\underline{z}$ 492) versus spectrum number.

This information is useful in deciding which spectra should be plotted and interpreted. Normally all spectra are plotted. Data are stored archivally on 9-track IBM-compatible magnetic tapes.

### 8.3.2 Spectral Interpretation

Identification of resolved components from data produced by all the mass spectrometer systems is achieved by comparing the fragmentation patterns of the unknown mass spectra to an eight major peak index of mass spectra (A9). Individual difficult unknowns are searched by the use of the Cornell University STIRS and PBM systems. Unknowns are also submitted to MSSS system for identification. When feasible, the identification of unknowns is confirmed by comparing the fragmentation pattern and elution temperatures for two different chromatographic columns (SE-30 and Carbowax SCOT capillaries) for the unknown and authentic compounds. The relationship between the boiling point of the identified compound and the elution temperature on a non-polar column (the order of elution of constituents is predictable in homologous series since the SE-30 SCOT capillary separates primarily on the basis of boiling point) is carefully considered in making structure assignments.

Mass spectral search programs are operational at the Triangle Universities Computation Center (TUCC). RTI maintains twice daily service to TUCC, which is a one-quarter mile distance from the RTI campus. Additional information about each magnetic tape containing mass spectra is entered directly into the TUCC job stream using a remote job entry processing. This is normally done at TUCC using one of the five terminals located within the Analytical Sciences Laboratory. The control information contains selected spectrum numbers with instructions to process entire GC runs. The computer program systems simultaneously compare either the entire library of 25,000 compounds or some subset of this library. The complete reports showing the best fits for each of the unknowns are produced at TUCC and printed out at the high speed terminals located on the RTI campus or TUCC.

### 8.3.3 Quantitative Analysis

In many cases the estimation of the level of pollutants by capillary gas chromatography in combination with mass spectrometry is not feasible utilizing only the total ion current monitor (See Figure A-3, for example). Since baseline resolution between peaks is not always achieved, we employ the techniques which have previously been developed under contract whereby full spectra are obtained during the chromatographic separation step and the selected ions are presented as mass fragmentograms using computer software programs which allow the possibility of deconvoluting constituents which were not resolved in the total ion current chromatogram (A6). Examples are depicted in Figures A-6 and A-7 which represent an ambient air sample with a TIC profile as in Figure A-3.

In our GC/MS/COMP systems we request from the dedicated computer mass fragmentograms for any combination of ions when full mass spectra have been obtained during chromatography. Thus selectivity is obtained by choosing characteristic ions for a particular organic substance. The ion intensity is plotted and the ion intensity vs. time is subsequently used for quantification. Also quantification with external standards is easily achieved using the intensity of the total ion current monitor or a characteristic ion in the mass spectrum of the external standard. Thus, we use mass fragmentography for the quantification of organics in ambient air when the total ion

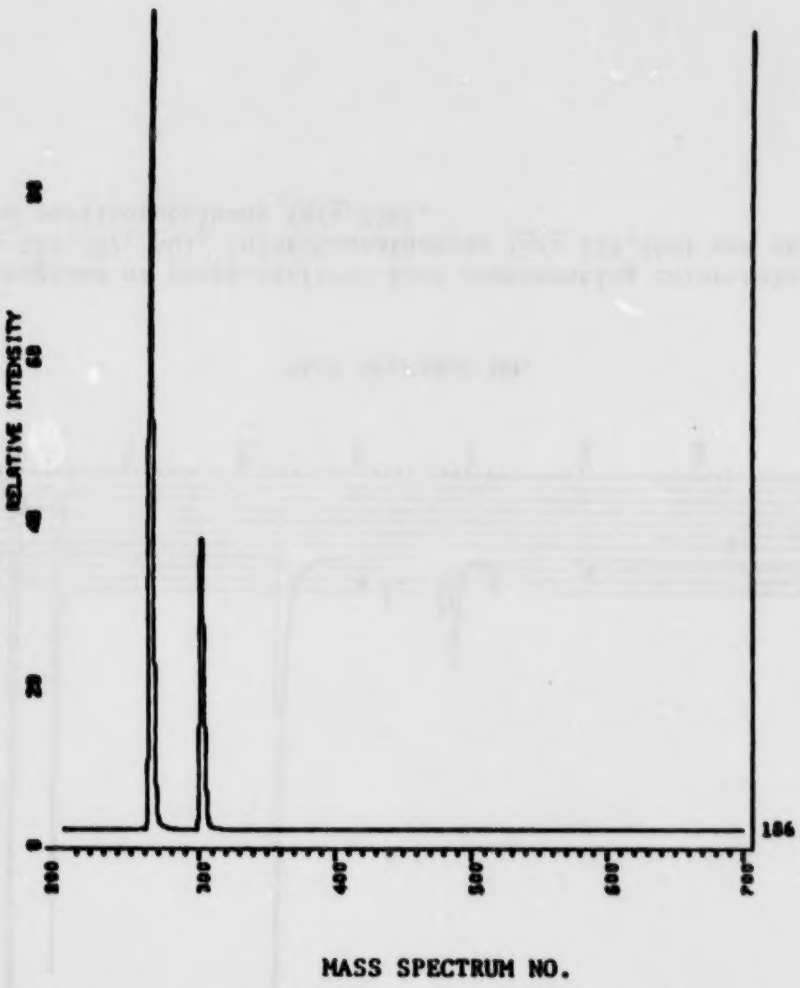


Figure A-6. Mass fragmentogram of ions characteristic of internal standards (perfluorobenzene, m/z 186 and perfluorotoluene, m/z 236 with a fragment at m/z 186) used in ambient air samples.

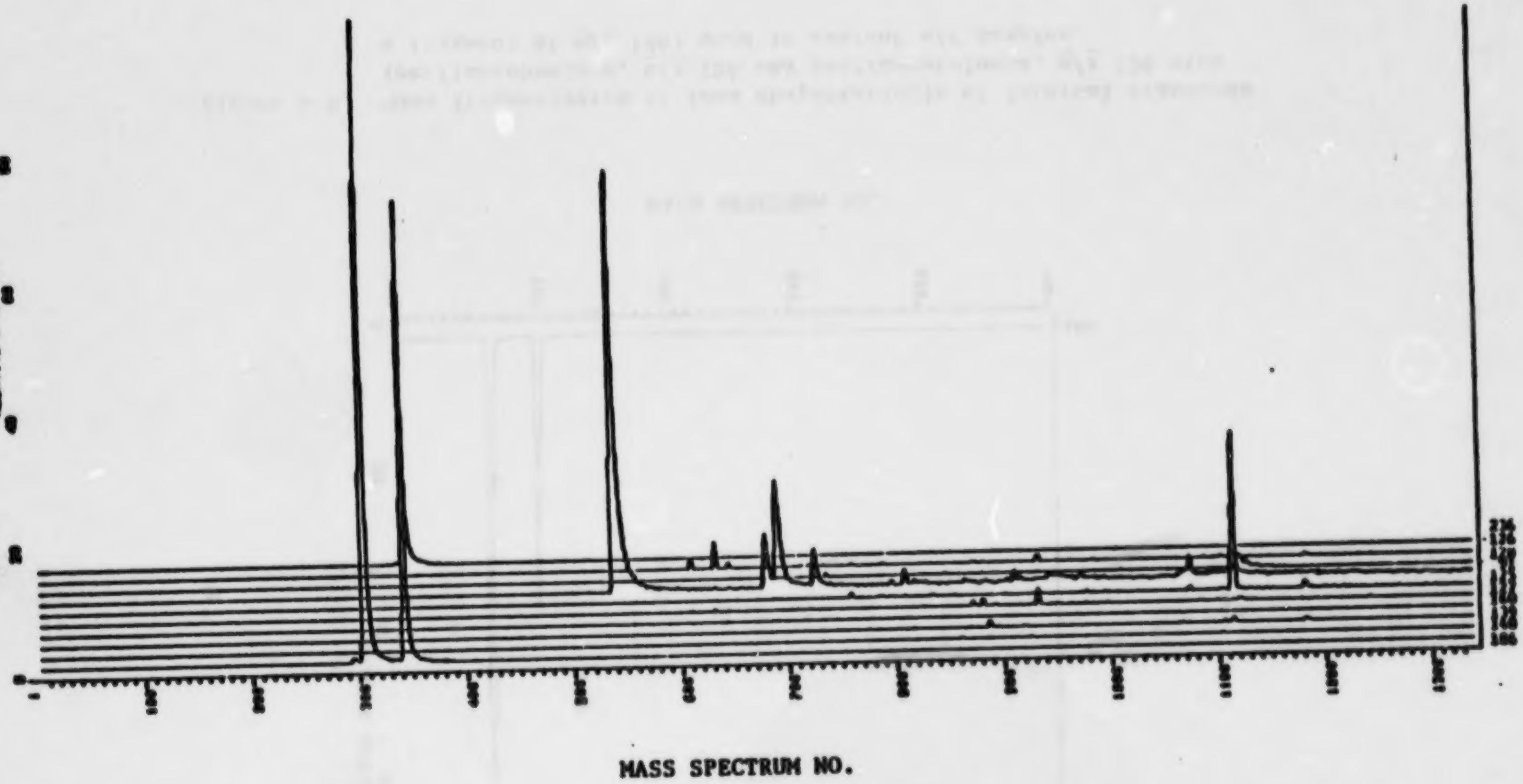


Figure A-7. Mass fragmentograms of characteristic ions representing chlorotoluene (m/z 91,126,128), dichlorotoluene (m/z 125,127,160), chlorobenzaldehyde (m/z 139,140) and standards perfluorobenzene (m/z 186) and perfluorotoluene (m/z 236).

current monitor is inadequate because of the lack of complete resolution between components in the mixture.

As described previously, the quantitation of constituents in ambient air samples is accomplished either by utilizing the total ion current monitor or, where necessary, mass fragmentograms. In order to eliminate the need to obtain complete calibration curves for each compound for which quantitative information is desired, we use the method of relative molar response (RMR) factors (A10). Successful use of this method requires information on the exact amount of standard added and the relationship of RMR (unknown) to the RMR (standards). The method of calculations is as follows:

$$(1)\quad RMR_{unknown/standard} = \frac{A_{unk}/Moles_{unk}}{A_{std}/Moles_{std}}$$

A = system response, height or area determined by integration or triangulation.

The value of RMR is determined from at least three independent analyses.

$$(2)\quad RMR_{unk/std} = \frac{A_{unk}/g_{unk}/GMW_{unk}}{A_{std}/g_{std}/GMW_{std}}$$

A = system response, as above
g = number of grams present
GMW = gram molecular weight

$$(3)\quad g_{unk} = \frac{A_{unk} \cdot GMW_{unk} \cdot g_{std}}{A_{std} \cdot GMW_{std} \cdot RMR_{unk/std}}$$

The standard can be added as an internal standard during sampling. Since, however, the volume of air taken to produce a given sample is accurately known, it is also possible and more practical to use an external standard where the standard is introduced into the cartridge prior to its analysis. Two standards, hexafluorobenzene and octafluorotoluene, are used for the purpose of calculating RMRs. From previous research it has been determined that the retention times for these two compounds are such that they elute from the glass capillary column (SE-30) at a temperature and retention time which does not interfere with the analysis of unknown compounds in ambient air samples.

Since the volume of air taken to produce a given sample is accurately known and an external standard is added to the sample, then the weight per cartridge and hence the concentration of the unknown can be determined. The approach for quantitating ambient air pollutants requires that the RMR be determined for each constituent of interest (before or after sample analysis). In this manner it is possible to obtain qualitative and quantitative information on the same sample with a minimum of effort.

## 9.0 References

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Written analytical protocol prepared 1/24/77, revised 4/79.

# APPENDIX B

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED ORGANICS IN WATER

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED ORGANICS IN WATER

## 1.0 Principle of the Method

Volatile compounds are recovered from an aqueous sample by warming the sample and purging an inert gas through the warm sample. The vapors are trapped on a Tenax cartridge and are then analyzed by thermal desorption interfaced to a GC/MS.

## 2.0 Range and Sensitivity

For a typical organic compound approximately 30 ng are required for mass spectral identification using high resolution glass capillary GC/MS analysis. A 100 ml water aliquot is used, so the limit is ~0.3 μg/l. The dynamic range for a purged sample is $\sim 10^4$, however, smaller samples may be purged and the range increased commensurately.

## 3.0 Interferences

Two possible types of interference must be considered: (1) material present in the sample which physically prevents the effective purge of the sample, and (2) a material which interferes with the analysis of the purged sample. In the former case, several techniques have been developed to handle such problems (e.g., foaming) by diluting and stirring the sample. The second case is minimized by the use of GC/MS for the analysis since unique combinations of m/z and retention time can be selected for most compounds. This permits the analysis of compounds even though chromatographic resolution is not obtained.

## 4.0 Precision and Accuracy

The purge and trap technique was validated using both $^{14}$C-labeled model compounds and "cold" model compounds. Results of these recovery studies are presented in Tables B-1 and B-2.

Based on these data, expected recoveries of purgeable halogenated organics from water are about 80% or better. Within the precision requirements of this study, these recovery values indicate that the method is essentially quantitative.

Table B-1. RECOVERY OF $^{14}$C-LABELED MODEL COMPOUNDS FROM WATER[a]

| | Compound | | | |
|---|---|---|---|---|
| | Chloroform | Carbon Tetrachloride | Chlorobenzene | Bromobenzene |
| 1 | 85.0 | 96.2 | 84.2 | 71.9 |
| 2 | 67.6 | 83.6 | 90.3 | 88.7 |
| 3 | 91.7 | 98.6 | 89.0 | 93.7 |
| 4 | 85.9 | 70.9 | 92.5 | 69.1 |
| 5 | 91.0 | 65.2 | 90.4 | 87.8 |
| 6 | 47.9 | 70.9 | 87.1 | 82.4 |
| 7 | 92.8 | 67.4 | 94.0 | 87.2 |
| 8 | 88.1 | - | 90.3 | 90.3 |
| Mean | 81.3 | 79.0 | 89.7 | 83.9 |
| ±S.D. | 15.7 | 14.0 | 3.1 | 8.9 |
| RSD (%) | 19.3 | 17.7 | 3.4 | 10.6 |

[a] 100 ml water spiked with 70,000-90,000 dpm of the model compound (∿1 μg).
SD = standard deviation
RSD = (SD/Mean) x 100

Table B-2. RECOVERY OF HALOGENATED HYDROCARBONS FROM DISTILLED WATER SPIKED FROM GAS MIXING BULB

| Compound | Amount spiked (μg) | % Recovery |  |  |  | Average ± Std. Dev. |
|---|---|---|---|---|---|---|
|  |  | 1 | 2 | 3 | 4 |  |
| methylene chloride | 1.33 | —[a] | —[a] | —[a] | —[a] |  |
| chloroform | 1.48 | 88.5 | 73.7 | 47.9 | 55.0 | 66.3 ± 18.4 |
| bromodichloromethane | 1.98 | 98.1 | 121 | 84.4 | 85.8 | 97.3 ± 16.9 |
| tetrachloroethylene | 1.70 | 71.4 | 65.9 | 64.2 | 101 | 75.6 ± 17.2 |
| chlorobenzene | 1.11 | 81.7 | 81.7 | 79.7 | 89.7 | 83.2 ± 4.4 |
| _m_-dichlorobenzene | 1.35 | 82.3 | 110 | 68.5 | 102 | 90.7 ± 18.8 |
| mean |  |  |  |  |  | 79.2 ± 23.9 |

[a] Not quantifiable because of background interferences.

## 5.0 Apparatus

### 5.1 Purge Apparatus

The apparatus required is shown in Figure B-1.

### 5.2 Sampling Cartridges

The sampling tubes are prepared by packing a 10 cm long x 1.5 cm i.d. glass tube containing 6.0 cm of 35/60 mesh Tenax GC (~1.6 g) using glass wool in the ends to provide support (B1-B5). Tenax GC is extracted in a Soxhlet extractor for a minimum of 24 hours each with methanol and then with pentane prior to preparation of cartridge samplers (B5). After purification, the Tenax GC sorbent is air dried, vacuum dried and then was meshed to provide a 35/60 particle size range. Cartridge samplers were then prepared and conditioned at 270°C with helium flow at 30 ml/min for 20 minutes. The conditioned cartridges were transferred to Kimax® (2.5 cm x 150 cm) culture tubes containing calcium sulfate desiccant which are immediately sealed using Teflon-lined caps to prevent contamination.

### 5.3 GC/MS/COMP

The volatile halogenated hydrocarbons purged from water are analyzed on either an LKB 2091 GC/MS with an LKB 2031 data system or a Varian MAT CH-7 GC/MS with a Varian 620/i data system. The sample, concentrated on a Tenax GC cartridge, is thermally desorbed using an inlet manifold system (B1-B3, B5). The operating conditions for the thermal desorption unit and the analysis of Tenax GC cartridges are given in Table B-3.

## 6.0 Procedure

### 6.1 Collection of Samples

Samples (120 ml) are obtained in triplicate from residential homeowners or other appropriate sources. The first sample is collected immediately and is analyzed for purgeable components. The second and third samples are taken after two minutes of continuous, moderate flow. One of these is analyzed for extractables and the other serves as a backup sample for either purgeables or extractables. Larger samples are optionally obtained from water and wastewater treatment plants.

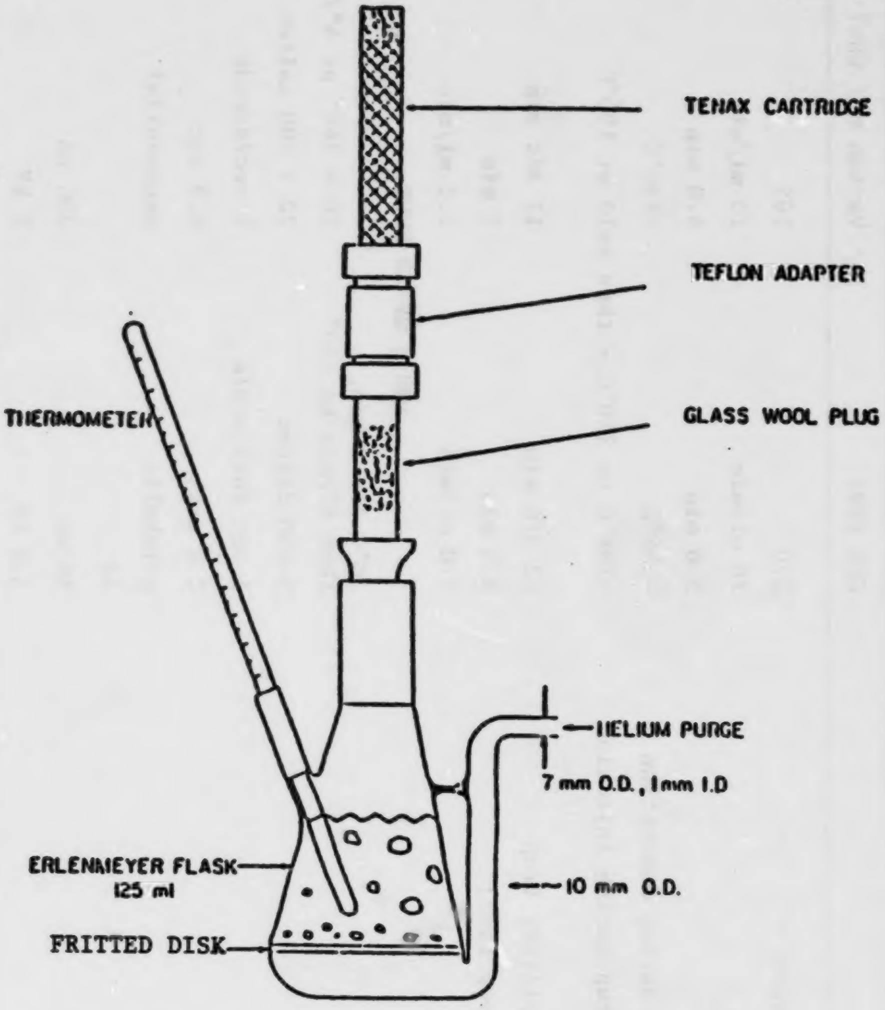
TENAX CARTRIDGE
TEFLON ADAPTER
GLASS WOOL PLUG
THERMOMETER
HELIUM PURGE
7 mm O.D., 1mm I.D
ERLENMEYER FLASK
125 ml
10 mm O.D.
FRITTED DISK

Figure B-1. Purge apparatus for water samples.

Table B-3. INSTRUMENTAL OPERATING CONDITIONS

| | LKB 2091 | Varian MAT CH-7 |
|---|---|---|
| Desorption chamber temperature | 270 | 265 |
| Desorption chamber He flow | 20 ml/min | 10 ml/min |
| Desorption time | 5.0 min | 8.0 min |
| Capillary Trap Temperature during desorption | -196°C | -196°C |
| Temperature of capillary trap during injection onto column | -196°C to 250°C - then held at 190°C | |
| Time of He flow through capillary trap | 12 3/4 min | 12 3/4 min |
| He flow through column [sweep time] | 9.5 min | 5 min |
| Carrier flow | 2.0 ml/min | 2.5 ml/min |
| Capillary column | 100 m SE-30 SCOT | |
| Column temperature | 30°C for 2 min, then 4°/min to 240° | 20 → 240° at 4°/min |
| Scan range | 5-490 dalton | 20 → 500 dalton |
| Scan rate | 1 sec full scale | 1 sec/decade |
| Scan cycle time | 2.4 sec | 4.5 sec |
| Scan mode | parabolic | exponential |
| Trap current | 4A | |
| Filament current | 50 μA | 200 μA |
| Accelerating volatage | 3.5 kV | 2 kV |

## 6.2 Purge of Volatile Organics

The water sample is cooled to ~4°C and a 100 ml aliquot transferred to the purge apparatus. The apparatus is assembled as depicted in Figure B-1 including the Tenax GC cartridge (1.5 cm diameter x 6.0 cm length). A carbon cartridge, 1.5 cm diameter x 4.0 cm length is connected to the effluent end of the Tenax cartridge to prevent contamination of the cartridge by laboratory vapors. The flask is wrapped with heating tape and the sample heated to 90°C. The sample is purged at 25 ml helium/min and 90°C for 30 minutes. The loaded cartridge is removed and stored in a culture tube containing 1-2 g of $CaSO_4$ desiccant for at least two hr. The desiccant is removed from the culture tube and the dry, loaded cartridge stored at -20°C.

## 6.3 Analysis of Sample Purged on Cartridge

The instrumental conditions for the analysis of halogenated hydrocarbons on the sorbent Tenax GC sampling cartridge is shown in Table B-3. The thermal desorption chamber and six-port valve are maintained at 270° and 200°C, respectively. The helium purge gas through the desorption chamber is adjusted to 15-20 ml/min. The nickel capillary trap at the inlet manifold is cooled with liquid nitrogen. In a typical thermal desorption cycle a sampling cartridge is placed in the preheated desorption chamber and helium gas is channeled through the cartridge to purge the vapors into the liquid nitrogen cooled nickel capillary trap. After desorption the six-port valve is rotated and the temperature on the capillary loop is rapidly raised; the carrier gas then introduces the vapors onto the high resolution GLC column. The glass capillary column is temperature programmed from 20° to 240°C at 4°/min and held at the upper limit for a minimum of 10 min. After all of the components have eluted from the capillary column the analytical column is then cooled to ambient temperature and the next sample is processed.

## 6.4 Quantitation

All data are acquired in the full scan mode. Quantitation of the halogenated compounds of interest is accomplished by utilizing selected ion plots, SIPs, which are plots of the intensity of specific ions (obtained from full scan data) vs. time. Using SIPs of ions characteristic of a given compound in conjunction with retention times permits quantitation of components of overlapping peaks. Two external standards, perfluorobenzene and perfluorotoluene, were added to each Tenax GC cartridge in known quantities just prior to analysis. In order to eliminate the need to construct complete calibration curves for each compound quantitated, the method of relative molar response (RMR) is used. In this method the relationship of the RMR as the unknown to the RMR of the standard is determined as follows:

$$\mathrm{RMR}_{\mathrm{unknown/standard}} = \frac{A_{\mathrm{unk}}/\mathrm{Moles}_{\mathrm{unk}}}{A_{\mathrm{std}}/\mathrm{Moles}_{\mathrm{std}}}$$

$$\mathrm{RMR}_{\mathrm{unk/std}} = \frac{A_{\mathrm{unk}}/g_{\mathrm{unk}}/\mathrm{GMW}_{\mathrm{unk}}}{A_{\mathrm{std}}/g_{\mathrm{std}}/\mathrm{GMW}_{\mathrm{std}}}$$

where $A$ = peak response of a selected ion,
$g$ = number of grams present, and
GMW = gram molecular weight

Thus, in the sample analyzed:

$$g_{\mathrm{unknown}} = \frac{(A_{\mathrm{unk}})(\mathrm{GMW}_{\mathrm{unk}})(g_{\mathrm{std}})}{(A_{\mathrm{std}})(\mathrm{GMW}_{\mathrm{std}})(\mathrm{RMR}_{\mathrm{unk/std}})}$$

The value of an RMR is determined from at least three independent analyses of standards of accurately known concentration prepared using a gas permeation system (B5). The precision of this method has been determined to be generally ±10 percent when replicate sampling cartridges are examined.

## 7.0 Quality Assurance Program

In addition to the validation procedures described above, an ongoing quality assurance program is required to assure the data quality. Quality control procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance procedures monitor the execution of the procedure and check data interpretation and calculations.

### 7.1 Quality Control

#### 7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each of the anticipated number of field samples. Blanks consist of 100 ml of prepurged, distilled water in the same type of sampling container as is used in the field. Controls consist of 100 ml of prepurged, distilled water spiked at 100-500 ng with the compounds listed in Table B-2. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

#### 7.1.2 Procedural Blanks and Controls

##### 7.1.2.1 Tenax GC

The purity of each batch of Tenax GC cartridges is checked by thermal desorption/GC/FID. An acceptable background is a judgement based upon the backgrounds obtained previously which exhibited no GC/MS artifacts.

##### 7.1.2.2 Purge Blanks

With each set of purge samples, a procedural blank is run. This consists of 100 ml of prepurged, distilled water which is purged under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion and storage.

##### 7.1.2.3 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a Tenax GC cartridge directly loaded with standards is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

### 7.2 Quality Assurance

#### 7.2.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

### 7.2.2 Documentation

#### 7.2.2.1 Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

#### 7.2.2.2 Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, location, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

#### 7.2.2.3 Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

#### 7.2.2.4 GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

## 8.0 References

B1. Pellizzari, E. D., Development of Method for Carcinogenic Vapor Analysis in Ambient Atmospheres, Publication No. EPA-650/2-74-121, Contract No. 68-02-1228, 148 pp., July, 1974.

B2. Pellizzari, E. D., Development of Analytical Technqiues for Measuring Ambient Atmospheric Carcinogenic Vapors. Publication No. EPA-600/2-76-076, Contract No. 68-02-1228, 185 pp., November, 1975.

B3. Pellizzari, E. D., J. E. Bunch, B. H. Carpenter and E. Sawicki, Environ. Sci. Tech., 9, 552 (1975).

B4. Pellizzari, E. D., B. H. Carpenter, J. E. Bunch and E. Sawicki, Environ. Sci. Tech., 9, 556 (1975).

B5. Pellizzari, E. D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors. Publication No. EPA 600/7-77-055, 288 pp., June, 1977.

Revised April, 1979

# APPENDIX C

## ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED HYDROCARBONS IN BLOOD

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED HYDROCARBONS IN BLOOD

## 1.0 Principle of the Method

Volatile compounds are recovered from a blood sample by warming the sample and purging an inert gas over the warm sample. The vapors are then trapped on a Tenax cartridge which is analyzed by thermal desorption interfaced to GC/MS.

## 2.0 Range and Sensitivity

For a typical organic compound approximately 30 ng are required for a mass spectral identification using high resolution glass capillary GC/MS analysis. Based on a 10 ml blood sample, the limit of detection is about 3 ng/ml or 3 ppb. The dynamic range for a purged sample is $\sim 10^4$, however, smaller samples may be purged and the range increased commensurately.

## 3.0 Interferences

Two possible types of interference must be considered: (1) material present in the sample which physically prevents the effective purge of the sample, and (2) a material which interferes with the analysis of the purged sample. In the former case, several techniques have been developed to handle such problems (e.g., foaming) by diluting and stirring the sample. The second case is minimized by the use of GC/MS for the analysis since unique combinations of m/z and retention time can be selected for most compounds. This permits the analysis of compounds even though chromatographic resolution is not obtained.

## 4.0 Precision and Accuracy

The purge and trap technique was validated using both $^{14}C$-labeled model compounds and "cold" model compounds. Results of these recovery studies are presented in Tables C-1 and C-2. Based on these data, expected recoveries of purgeable halogenated organics from blood are about 80% or better. Within the precision requirements of this study, these recovery values indicate that the method is essentially quantitative.

Table C-1. PERCENT RECOVERY OF $^{14}$C-LABELED COMPOUND FROM BLOOD[a]

| Compound | Mass Loading (μg) | dpm Loaded | % Recovery | Average % Recovery |
|---|---|---|---|---|
| chloroform | 1.46 | 91755.1 | 94.2 | 93.7 |
| | | | 93.1 | |
| | | | 46.5[b] | |
| carbon tetrachloride | 0.785 | 77902.3 | 92.6 | 89.4 |
| | | | 92.2 | |
| | | | 83.5 | |
| chlorobenzene | 1.12 | 89538.1 | 43.5[b] | 90.1 |
| | | | 88.0 | |
| | | | 92.2 | |
| bromobenzene | 1.21 | 84493.9 | 77.1 | 80.6 |
| | | | 74.4 | |
| | | | 90.3 | |

[a] Headspace purge of whole blood (25 ml) diluted 1:1 with distilled water and purged in 100 ml 3-neck purge flasks at 50°C for 90 min with helium at 25 ml/min.

[b] Leaking desorption chamber cap; not included in average.

Table C-2. RECOVERY OF HALOGENATED HYDROCARBONS FROM HUMAN BLOOD SPIKED FROM GAS MIXING BULB

| Compound | Amount spiked (μg) | % Recovery | | | | Average ± Std. Dev. |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | |
| methylene chloride | 7.96 | 105 | 116 | —[a] | —[a] | 111 ± 7.8 |
| chloroform | 8.16 | 84.6 | 145 | 139 | —[a] | 123 ± 33.1 |
| bromodichloromethane | 10.4 | 105 | 159 | 94.9 | 94.9 | 113 ± 30.7 |
| tetrachloroethylene | 8.52 | 108 | 121 | 82.8 | 97.5 | 99.8 ± 17.9 |
| chlorobenzene | 5.53 | 108 | 120 | 85.6 | 78.6 | 98.1 ± 19.3 |
| m-dichlorobenzene | 6.44 | 85.1 | 101 | 74.6 | 84.5 | 86.3 ± 10.9 |
| mean | | | | | | 104.3 ± 22.7 |

[a] Not quantified because of background interferences.

## 5.0 Apparatus

### 5.1 Sampling Apparatus

Vacutainers "suitable for GC", Venoject L 428 (Kimble, or other high purity vacutainers; ice bath; disposable Pasteur pipettes and bulbs, and cleaned and oven-treated shell vials with Teflon-lined screw caps.

### 5.2 Purge Apparatus

The apparatus required is shown in Figure C-1.

### 5.3 Tenax Cartridges

The sampling tubes are prepared by packing a 10 cm long x 1.5 cm i.d. glass tube containing 6.0 cm of 35/60 mesh Tenax GC (~1.6 g), using glass wool in the ends to provide support (C1-C5). Tenax GC is extracted in a Soxhlet extractor for a minimum of 24 hours each with methanol and then with pentane prior to preparation of cartridge samplers (C1). After purification, the Tenax GC sorbent is air-dried, vacuum dried and then meshed to provide a 35/60 particle size range. Cartridge samplers are then prepared and conditioned at 270°C with helium flow at 30 ml/min for 20 minutes. The conditioned cartridges are transferred to Kimax® (2.5 cm x 150 cm) culture tubes which are immediately sealed using Teflon-lined caps to prevent contamination.

### 5.4 GC/MS/COMP

The volatile halogenated hydrocarbons purged from water are analyzed on either an LKB 2091 GC/MS with an LKB 2031 data system or a Varian MAT CH-7 GC/MS with a Varian 620/i data system. The sample, concentrated on a Tenax GC cartridge is thermally desorbed using an inlet manifold system (C1-C3, C5). The operating conditions for the thermal desorption unit and the analysis of Tenax GC cartridges are given in Table C-3.

## 6.0 Procedure

### 6.1 Collection of Samples

Blood samples are collected in replicate 10 ml vacutainer tubes containing an anticoagulant. Using a qualified phlebotomist, the samples are collected by brachial venipuncture. Glass syringes represent the optimal collection device, since no polymeric material which may contaminate the sample comes in contact with the blood. However, sterilization of large

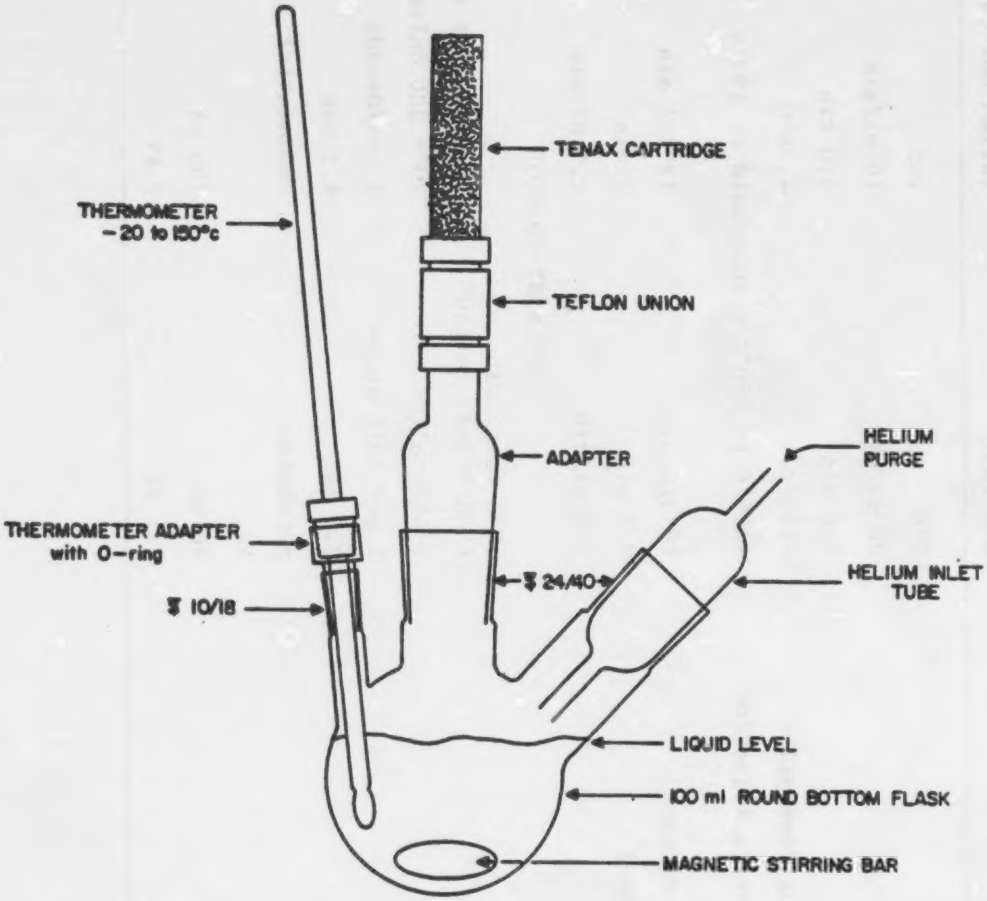
TENAX CARTRIDGE
THERMOMETER
-20 to 150°c
TEFLON UNION
ADAPTER
HELIUM
PURGE
THERMOMETER ADAPTER
with O-ring
$ 24/40
HELIUM INLET
TUBE
$ 10/18
LIQUID LEVEL
100 ml ROUND BOTTOM FLASK
MAGNETIC STIRRING BAR

Figure C-1. Headspace purge apparatus for blood, urine, and tissue samples.

## Table C-3. INSTRUMENTAL OPERATING CONDITIONS

| | LKB 2091 | Varian MAT CH-7 |
|---|---|---|
| Desorption chamber temperature | 270 | 265 |
| Desorption chamber He flow | 20 ml/min | 10 ml/min |
| Desorption time | 5.0 min | 8.0 min |
| Capillary Trap Temperature during desorption | -196°C | -196°C |
| Temperature of capillary trap during injection onto column | -196°C to 250°C - then held at 190°C | |
| Time of He flow through capillary trap | 12 3/4 min | 12 3/4 min |
| He flow through column [sweep time] | 9.5 min | 5 min |
| Carrier flow | 2.0 ml/min | 2.5 ml/min |
| Capillary column | 100 m SE-30 SCOT | |
| Column temperature | 30°C for 2 min, then 4°/min to 240° | 20 → 240° at 4°/min |
| Scan range | 5-490 dalton | 20 → 500 dalton |
| Scan rate | 1 sec full scale | 1 sec/decade |
| Scan cycle time | 2.4 sec | 4.5 sec |
| Scan mode | parabolic | exponential |
| Trap current | 4A | |
| Filament current | 50 µA | 200 µA |
| Accelerating volatage | 3.5 kV | 2 kV |

numbers of glass syringes in the field is not practical, so vacutainers will be used.

Possible contamination by permeation through the rubber septum caps of the vacutainers is a cause for concern. Teflon-lined vacutainers are not available but manufacturers recommended special vacutainers "suitable for GC" (Venoject L 428, Kimble). Validation experiments for benzene under a separate contract (EPA No. 68-01-3849, Task I) found the background of these tubes to be acceptable.

Following collection, shipping and storage procedures must assure that the purgeable halogenated organics remain intact in the blood sample. Leakage of the vacutainer caps has been observed and permeation through the cap material is suspected. Accordingly, these containers are not suitable for storage. In the procedure used for this project, the blood sample is chilled and transferred to a clean shell vial with a Teflon-lined screw cap. The cap is then taped shut. This procedure has been validated and field tested on EPA Contract No. 68-01-3849, Task I.

## 6.2 Purge of Volatile Organics

An aliquot of whole blood, chilled to 4°C is measured into the purge flask in Figure C-1. Prepurged, distilled water is added to the sample to dilute it to about 50 ml; and a stirring bar added. The apparatus is assembled, stirring started, the temperature raised to 50°C, and the helium flow started at 25 ml/min. After 90 min, the purge is terminated, the apparatus disassembled, and the Tenax cartridge stored in a Kimax culture tube with calcium sulfate desiccant in a freezer until it is ready for analysis.

## 6.3 Analysis of Sample Purged on Cartridge

The instrumental conditions for the analysis of halogenated hydrocarbons of the sorbent Tenax GC sampling cartridge is shown in Table C-3. The thermal desorption chamber and six-port valve are maintained at 270° and 200°C, respectively. The helium purge gas through the desorption chamber is adjusted to 15-20 ml/min. The nickel capillary trap at the inlet manifold is cooled with liquid nitrogen. In a typical thermal desorption cycle a sampling cartridge is placed in the preheated desorption chamber and helium gas is channeled through the cartridge to purge the vapors into the liquid nitrogen cooled nickel capillary trap. After desorption the six-port valve is rotated and the temperature on the capillary loop is rapidly raised; the carrier gas then introduces the vapors onto the high resolution GLC column. The glass capillary column is temperature programmed from 20° to 240°C at 4°/min and held at the upper limit for a minimum of 10 min. After all of the components have eluted from the capillary column the analytical column is then cooled to ambient temperature and the next sample is processed (C2).

### 6.4 Quantitation

All data are acquired in the full scan mode. Quantitation of the halogenated compounds of interest is accomplished by utilizing selected ion plots, SIPs, which are plots of the intensity of specific ions (obtained from full scan data) vs. time. Using SIPs of ions characteristic of a given compound in conjunction with retention times permits quantitation of components of overlapping peaks. Two external standards, perfluorobenzene and perfluorotoluene, were added to each Tenax GC cartridge in known quantities just prior to analysis. In order to eliminate the need to construct complete calibration curves for each compound quantitated, the method of relative molar response (RMR) is used. In this method the relationship of the RMR of the unknown to the RMR of the standard is determined as follows:

$$\mathrm{RMR}_{\mathrm{unknown}} = \frac{A_{\mathrm{unk}}/\mathrm{Moles}_{\mathrm{unk}},}{A_{\mathrm{std}}/\mathrm{Moles}_{\mathrm{std}}}$$

$$\mathrm{RMR}_{\mathrm{unk/std}} = \frac{A_{\mathrm{unk}}/g_{\mathrm{unk}}/\mathrm{GMW}_{\mathrm{unk}}}{A_{\mathrm{std}}/g_{\mathrm{std}}/\mathrm{GMW}_{\mathrm{std}}}$$

where $A$ = peak response of a selected ion,
$g$ = number of grams present, and
GMW = gram molecular weight.

Thus, in the sample being analyzed:

$$g_{\mathrm{unknown}} = \frac{(A_{\mathrm{unk}})(\mathrm{GMW}_{\mathrm{unk}})(g_{\mathrm{std}})}{(A_{\mathrm{std}})(\mathrm{GMW}_{\mathrm{std}})(\mathrm{RMR}_{\mathrm{unk/std}})}.$$

The value of an RMR is determined from at least three independent analyses of standards of accurately known concentration prepared using a gas permeation system (C5). The precision of this method has been determined to be generally ±10 percent when replicate sampling cartridges are examined.

## 7.0 Quality Assurance Program

In addition to the validation procedures described above, an ongoing quality assurance program is required to assure the data quality. Quality control procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance procedures monitor the execution of the procedure and check data interpretation and calculations.

### 7.1 Quality Control

#### 7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each of the anticipated number of field samples. Blanks consist of 10 ml of locally obtained blood plasma in the same type of sampling container as is used in the field. Controls consist of 10 ml of locally obtained blood plasma spiked with 10 ng each of the compounds listed in Table C-2. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

#### 7.1.2 Procedure of Blanks and Controls

##### 7.1.2.1 Tenax GC

The purity of each batch of Tenax GC cartridges is checked by thermal desorption/GC/FID. An "acceptable background" is a judgement based upon the backgrounds obtained previously which exhibited no GC/MS artifacts.

##### 7.1.2.2 Purge Blanks

With each set of purge samples, a procedural blank is run. This consists of 50 ml of prepurged distilled water which is purged under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion and storage.

##### 7.1.2.3 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a Tenax GC cartridge directly loaded with standards is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

## 7.2 Quality Assurance

### 7.2.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

### 7.2.2 Documentation

### 7.2.2.1 Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

### 7.2.2.2 Sampling Protocol Sheets

When a sample is collected, a Sampling Protocol Sheet is filled in which contains a discrete sample code which identifies project number, area, site, location, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

### 7.2.2.3 Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

### 7.2.2.4 GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data have been produced.

## 8.0 References

C1. Pellizzari, E. D., Development of Method for Carcinogenic Vapor Analysis in Ambient Atmospheres. Publication No. EPA-650/2-74-121, Contract No. 68-02-1228, 148 pp., July, 1974.

C2. Pellizzari, E. D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors. Publication No. EPA-600/2-76-076, Contract No. 68-02-1228, 185 pp., November, 1975.

C3. Pellizzari, E. D., J. E. Bunch, B. H. Carpenter and E. Sawicki, Environ. Sci. Tech., 9, 552 (1975).

C4. Pellizzari, E. D., B. H. Carpenter, J. E. Bunch and E. Sawicki, Environ. Sci. Tech., 9, 556 (1975).

C5. Pellizzari, E. D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors, Publication No. EPA-600/7-77-055, 288 pp., June 1977.

Revised April, 1979.

# APPENDIX D

## ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED ORGANICS IN URINE

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED ORGANICS IN URINE

## 1.0 Principle of the Method

Volatile compounds are recovered from a urine sample by warming the sample and purging an inert gas over the warm sample. The vapors are then trapped on a Tenax cartridge and subsequently can be analyzed by thermal desorption interfaced to GC/MS.

## 2.0 Range and Sensitivity

For a typical organic compound approximately 30 ng are required for mass spectral identification using high resolution glass capillary GC/MS analysis. Based on a 25 ml urine sample, a limit of detection of about 1.2 μg/l (1.2 ppb) may be expected. The dynamic range for a purged sample is $\sim 10^4$, however, smaller samples may be purged and the range increased commensurately.

## 3.0 Interferences

Two possible types of interference must be considered: (1) material present in the sample which physically prevents the effective purge of the sample, and (2) a material which interferes with the analysis of the purged sample. In the former case, several techniques have been developed to handle such problems (e.g., foaming) by diluting and stirring the sample. The second case is minimized by the use of GC/MS for the analysis since unique combinations of m/z and retention time can be selected for most compounds. This permits the analysis of compounds even though chromatographic resolution is not obtained.

## 4.0 Precision and Accuracy

The purge and trap technique was validated using both $^{14}C$-labeled model compounds and "cold" model compounds. Results of these recovery studies are presented in Tables D-1 and D-2.

Based on these data, expected recoveries of purgeable halogenated organics from urine are about 80% or better. Within the precision requirements of this study, these recovery values indicate that the method is essentially quantitative.

Table D-1. PERCENT RECOVERY OF $^{14}$C-LABELED COMPOUNDS FROM URINE

| Compound | Mass Loaded (μg) | dpm Loaded | % Recovery | Average % Recovery |
|---|---|---|---|---|
| chloroform | 1.50 | 94237.0 | 83.9<br>84.3<br>85.2 | 84.5 |
| carbon tetrachloride | 0.809 | 80278.6 | 57.2<br>66.7<br>67.1 | 63.7 |
| chlorobenzene | 1.12 | 89589.9 | 89.9<br>87.8<br>90.2 | 89.3 |
| bromobenzene | 0.0127 | 885.4 | 147<br>114<br>107 | 123 |

Table D-2. RECOVERY OF HALOGENATED HYDROCARBONS FROM HUMAN URINE SPIKED FROM GAS MIXING BULB

| Compound | Amount spiked (µg) | % Recovery |  |  |  | Average ± Std. Dev. |
|---|---|---|---|---|---|---|
|  |  | 1 | 2 | 3 | 4 |  |
| methylene chloride | 6.96 | 80.6 | —[a] | 41.3 | 21.7 | 47.9 ± 30.0 |
| chloroform | 8.16 | 107 | 72.6 | 89.5 | 44.8 | 78.5 ± 26.5 |
| bromodichloromethane | 10.2 | 123 | 88.2 | 126 | 80.8 | 105 ± 23.3 |
| tetrachloroethylene | 8.44 | 85.2 | 61.2 | 84.4 | 57.1 | 72.0 ± 14.9 |
| chlorobenzene | 5.58 | 103 | 77.1 | 92.6 | 71.9 | 86.2 ± 14.2 |
| m-dichlorobenzene | 7.54 | 73.6 | 70.5 | 90.1 | 80.7 | 78.7 ± 8.7 |
| mean |  |  |  |  |  | 79.2 ± 24.3 |

[a] Not quantified because of background interferences.

## 5.0 Apparatus

### 5.1 Sampling

Urine samples are collected in 120 ml cleaned and oven-treated glass bottles.

### 5.2 Purge Apparatus

The apparatus required is shown in Figure D-1.

### 5.3 Tenax Cartridges

The sampling tubes are prepared by packing a 10 cm long x 1.5 cm i.d. glass tube containing 6.0 cm of 35/60 mesh Tenax GC (~1.6 g) using glass wool in the ends to provide support (D1-D5). Tenax GC is extracted in a Soxhlet extractor for a minimum of 24 hours each with methanol and then with pentane prior to preparation of cartridge samplers (D5). After purification, the Tenax GC sorbent is air dried, vacuum dried and then was meshed to provide a 35/60 particle size range. Cartridge samplers were then prepared and conditioned at 270°C with helium flow at 30 ml/min for 20 minutes. The conditioned cartridges were transferred to Kimax® (2.5 cm x 150 cm) culture tubes which are immediately sealed using Teflon-lined caps to prevent contamination.

### 5.4 GC/MS/COMP

The volatile halogenated hydrocarbons purged from water are analyzed on either an LKB 2091 GC/MS with an LKB 2031 data system or a Varian MAT CH-7 GC/MS with a Varian 620/i data system. The sample, concentrated on a Tenax GC cartridge, is thermally desorbed using an inlet manifold system (D1-D3, D5). The operating conditions for the thermal desorption unit and the analysis of Tenax GC cartridges are given in Table D-3.

## 6.0 Procedure

### 6.1 Collection of Samples

Each participant is provided with a clean 120 ml (4 oz.) bottle and asked to collect the first urine sample in the morning. In addition, spot urine samples are collected from selected participants when tissue, breath and blood samples are collected. These samples serve both as backups and to determine individual variability.

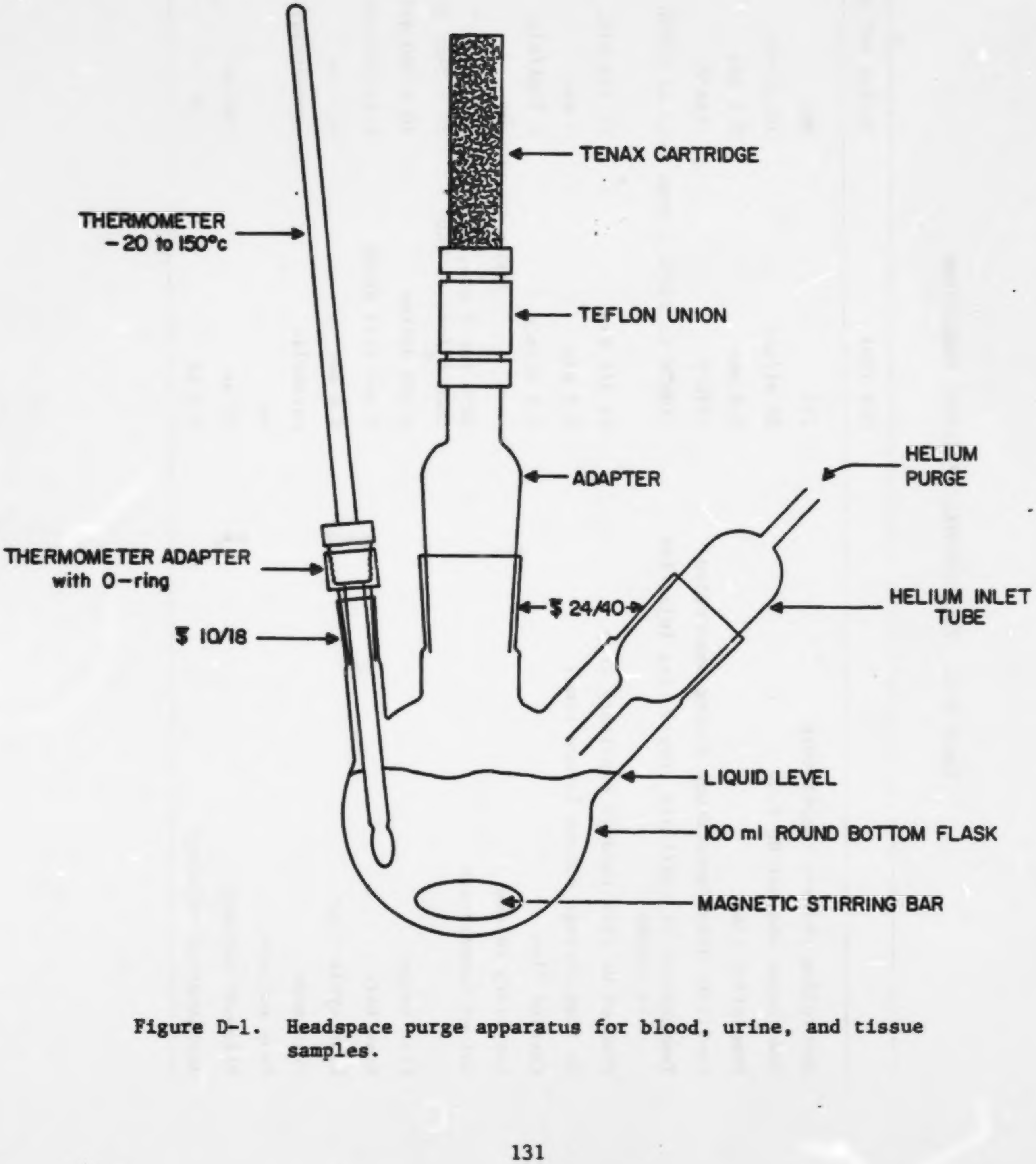
TENAX CARTRIDGE
THERMOMETER
-20 to 150°c
TEFLON UNION
ADAPTER
HELIUM
PURGE
THERMOMETER ADAPTER
with O-ring
$ 24/40
HELIUM INLET
TUBE
$ 10/18
LIQUID LEVEL
100 ml ROUND BOTTOM FLASK
MAGNETIC STIRRING BAR

Figure D-1. Headspace purge apparatus for blood, urine, and tissue samples.

## Table D-3. INSTRUMENTAL OPERATING CONDITIONS

| | LKB 2091 | Varian MAT CH-7 |
|---|---|---|
| Desorption chamber temperature | 270 | 265 |
| Desorption chamber He flow | 20 ml/min | 10 ml/min |
| Desorption time | 5.0 min | 8.0 min |
| Capillary Trap Temperature during desorption | -196°C | -196°C |
| Temperature of capillary trap during injection onto column | -196°C to 250°C - then held at 190°C | |
| Time of He flow through capillary trap | 12 3/4 min | 12 3/4 min |
| He flow through column [sweep time] | 9.5 min | 5 min |
| Carrier flow | 2.0 ml/min | 2.5 ml/min |
| Capillary column | 100 m SE-30 SCOT | |
| Column temperature | 30°C for 2 min, then 4°/min to 240° | 20 → 240° at 4°/min |
| Scan range | 5-490 dalton | 20 → 500 dalton |
| Scan rate | 1 sec full scale | 1 sec/decade |
| Scan cycle time | 2.4 sec | 4.5 sec |
| Scan mode | parabolic | exponential |
| Trap current | 4A | |
| Filament current | 50 µA | 200 µA |
| Accelerating volatage | 3.5 kV | 2 kV |

### 6.2 Purge of Volatile Organics

A 25 ml aliquot of urine, chilled to 4°C is measured into the purge flask in Figure D-1; purged, distilled water is added to the sample to dilute it to about 50 ml; and a stirring bar added. The apparatus is assembled, stirring started, the temperature raised to 50°C, and the helium flow started at 25 ml/min. After 90 min, the purge is terminated, the apparatus disassembled, and the Tenax cartridge stored in a Kimax culture tube with calcium sulfate desiccant in a freezer until it is ready for analysis.

### 6.3 Analysis of Sample Purged on Cartridge

The instrumental conditions for the analysis of halogenated hydrocarbons of the sorbent Tenax GC sampling cartridge is shown in Table D-3. The thermal desorption chamber and six-port valve are maintained at 270° and 200°C, respectively. The helium purge gas through the desorption chamber is adjusted to 15-20 ml/min. The nickel capillary trap at the inlet manifold is cooled with liquid nitrogen. In a typical thermal desorption cycle a sampling cartridge is placed in the preheated desorption chamber and helium gas is channeled through the cartridge to purge the vapors into the liquid nitrogen cooled nickel capillary trap. After desorption the six-port valve is rotated and the temperature on the capillary loop is rapidly raised; the carrier gas then introduces the vapors onto the high resolution GLC column. The glass capillary column is temperature programmed from 20° to 240°C at 4°/min and held at the upper limit for a minimum of 10 min. After all of the components have eluted from the capillary column the analytical column is then cooled to ambient temperature and the next sample is processed.

### 6.4 Quantitation

All data are acquired in the full scan mode. Quantitation of the halogenated compounds of interest is accomplished by utilizing selected ion plots, SIPs, which are plots of the intensity of specific ions (obtained from full scan data) vs. time. Using SIPs of ions characteristic of a given compound in conjunction with retention times permits quantitation of components of overlapping peaks. Two external standards, perfluorobenzene and perfluorotoluene, were added to each Tenax GC cartridge in known quantities just prior to analysis. In order to eliminate the need to construct complete calibration curves for each compound quantitated, the method of relative molar response (RMR) is used. In this method the relationship of the RMR as the unknown to the RMR of the standard is determined as follows:

$$\mathrm{RMR}_{\mathrm{unknown/standard}} = \frac{A_{\mathrm{unk}}/\mathrm{Moles}_{\mathrm{unk}}}{A_{\mathrm{std}}/\mathrm{Moles}_{\mathrm{std}}}$$

$$\mathrm{RMR}_{\mathrm{unk/std}} = \frac{A_{\mathrm{unk}}/g_{\mathrm{unk}}/\mathrm{GMW}_{\mathrm{unk}}}{A_{\mathrm{std}}/g_{\mathrm{std}}/\mathrm{GMW}_{\mathrm{std}}}$$

where A = peak response of a selected ion,
g = number of grams present, and
GMW = gram molecular weight

Thus, in the sample analyzed:

$$g_{\mathrm{unknown}} = \frac{(A_{\mathrm{unk}})(\mathrm{GMW}_{\mathrm{unk}})(g_{\mathrm{std}})}{(A_{\mathrm{std}})(\mathrm{GMW}_{\mathrm{std}})(\mathrm{RMR}_{\mathrm{unk/std}})}$$

The value of an RMR is determined from at least three independent analyses of standards of accurately known concentration prepared using a gas permeation system (D5). The precision of this method has been determined to be generally ±10 percent when replicate sampling cartridges are examined.

## 7.0 Quality Assurance Program

In addition to the validation procedures described above, an ongoing quality assurance program is required to assure the data quality. Quality control procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance procedures monitor the execution of the procedure and check data interpretation and calculations.

## 7.1 Quality Control

### 7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each of the anticipated number of field samples. Blanks consist of 120 ml of prepurged, distilled water in the same type of sampling container as is used in the field. Controls consist of 120 ml urine spiked with 100-500 ng each of the compounds listed in Table D-2. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

#### 7.1.2 Procedural Blanks and Controls

##### 7.1.2.1 Tenax GC

The purity of each batch of Tenax GC cartridges is checked by thermal desorption/GC/FID. An acceptable background is a judgement based upon the backgrounds obtained previously which exhibited no GC/MS artifacts.

##### 7.1.2.2 Purge Blanks

With each set of purge samples, a procedural blank is run. This consists of 50 ml of prepurged, distilled water which is purged under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion and storage.

##### 7.1.2.3 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one GC sample is run each working day. In addition, a Tenax GC cartridge directly loaded with standards is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

### 7.2 Quality Assurance

#### 7.2.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

### 7.2.2 Documentation

#### 7.2.2.1 Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

#### 7.2.2.2 Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, location, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

#### 7.2.2.3 Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

#### 7.2.2.4 GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

## 8.0 References

D1. Pellizzari, E. D., Development of Method for Carcinogenic Vapor Analysis in Ambient Atmospheres, Publication No. EPA-650/2-74-121, Contract No. 68-02-1228, 148 pp., July, 1974.

D2. Pellizzari, E. D., Development of Analytical Technqiues for Measuring Ambient Atmospheric Carcinogenic Vapors. Publication No. EPA-600/2-76-076, Contract No. 68-02-1228, 185 pp., November, 1975.

D3. Pellizzari, E. D., J. E. Bunch, B. H. Carpenter and E. Sawicki, Environ. Sci. Tech., 9, 556 (1975).

D4. Pellizzari, E. D., B. H. Carpenter, J. E. Bunch and E. Sawicki, Environ. Sci. Tech., 9, 556 (1975).

D5. Pellizzari, E. D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors. Publication No. EPA 600/7-77-055, 288 pp., June, 1977.

Revised April, 1979

# APPENDIX E

## ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED ORGANICS IN TISSUE

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED ORGANICS IN TISSUE

## 1.0 Principle of the Method

Volatile compounds are recovered from a tissue sample by macerating with water, warming the sample and purging an inert gas over the warm sample. The vapors are then trapped on a Tenax cartridge which is analyzed by thermal desorption interfaced to GC/MS.

## 2.0 Range and Sensitivity

For a typical organic compound approximately 30 ng are required for mass spectral identification using high resolution glass capillary GC/MS analysis. Based on a 5 g tissue sample, the limit of detection of about 6 ng/g (6 ppb) would be typical. The dynamic range for a purged sample is $\sim 10^4$, however, smaller samples may be purged and the range increased commensurately.

## 3.0 Interferences

Two possible types of interference must be considered: (1) material present in the sample which physically prevents the effective purge of the sample, and (2) a material which interferes with the analysis of the purged sample. In the former case, several techniques have been developed to handle such problems (e.g., foaming) by diluting and stirring the sample. The second case is minimized by the use of GC/MS for the analysis since unique combinations of $m/z$ and retention time can be selected for most compounds. This permits analysis of compounds even though chromatographic resolution is not obtained.

## 4.0 Precision and Accuracy

The purge and trap technique was validated using "cold" model compounds. Results of these recovery studies are presented in Table E-1. Based on these data, expected recoveries of purgeable halogenated organics from tissue are about 50%.

Great difficulty was encountered in quantitative introduction of a representative fortified sample into the container for analysis. Consequently variations in recovery may be attributed to losses during tissue maceration and transfer.

Table E-1. RECOVERY OF HALOGENATED HYDROCARBON FROM HUMAN ADIPOSE TISSUE.

| Compound | % Recovery |  |  |  |  |
|---|---|---|---|---|---|
|  | 1 | 2 | 3 | 4 | Average ± S.D. |
| methylene chloride | —[a] | 78.2 | 69.8 | 92.8 | 80.3 ± 11.6 |
| chloroform | —[a] | 62.3 | 14.8 | 59.7 | 45.6 ± 26.7 |
| bromodichloromethane | 64.4 | 15.5 | 13.4 | 49.4 | 35.7 ± 25.3 |
| tetrachloroethylene | 39.4 | 15.2 | 112 | 40.6 | 51.8 ± 41.8 |
| chlorobenzene | 21.5 | 6.8 | 7.3 | 17.9 | 13.4 ± 7.5 |
| *m*-dichlorobenzene | 82.1 | —[a] | 56.5 | 30.8 | 56.5 ± 25.7 |
| Mean |  |  |  |  | 45.3 ± 30.8 |

[a] Not quantified due to peak interference.

The tissue sample analysis procedure has been partially validated. Due to some low recovery values listed in Table E-1, the difficulties in handling tissue during the purge, and (most significantly) the lack of control over sampling and storage prior to receipt by RTI; the analysis of tissue samples of a purgeable halogenated organics must be regarded as semiquantitative.

## 5.0 Apparatus

### 5.1 Sampling Apparatus

Samples must be collected and stored with a minimum potential for contamination or loss of volatile components. Plastic or "rubber" containers can leach contaminents into the sample. In addition, purgeable organics can permeate through plastic, either representing a loss or an artifact from air pollutants (e.g. freons from a freezer compressor).

Samples should be collected with minimum exposure to plastic or rubber. They should be stored in cleaned, oven-treated glass jars sealed with either Teflon or foil-lined caps.

### 5.2 Purge Apparatus

The apparatus required is shown in Figure E-1.

### 5.3 Tenax Cartridges

The sampling tubes are prepared by packing a 10 cm long x 1.5 cm i.d. glass tube containing 6.0 cm of 35/60 mesh Tenax GC (~1.6 g), using glass wool in the ends to provide support (E1-E5). Tenax GC is extracted in a Soxhlet extractor for a minimum of 24 hours each with methanol and then with pentane prior to preparation of cartridge samplers (E5). After purification, the Tenax GC sorbent is air-dried, vacuum dried and then meshed to provide a 35/60 particle size range. Cartridge samplers are then prepared and conditioned at 270°C with helium flow at 30 ml/min for 20 minutes. The conditioned cartridges are transferred to Kimax® (2.5 cm x 150 cm) culture tubes which are immediately sealed using Teflon-lined caps to prevent contamination.

### 5.4 GC/MS/COMP

The volatile halogenated hydrocarbons purged from water are analyzed on either an LKB 2091 GC/MS with an LKB 2031 data system or a Varian MAT CH-7 GC/MS with a Varian 620/i data system. The sample, concentrated on a Tenax GC cartridge, is thermally desorbed using an inlet manifold system (E1-E3,

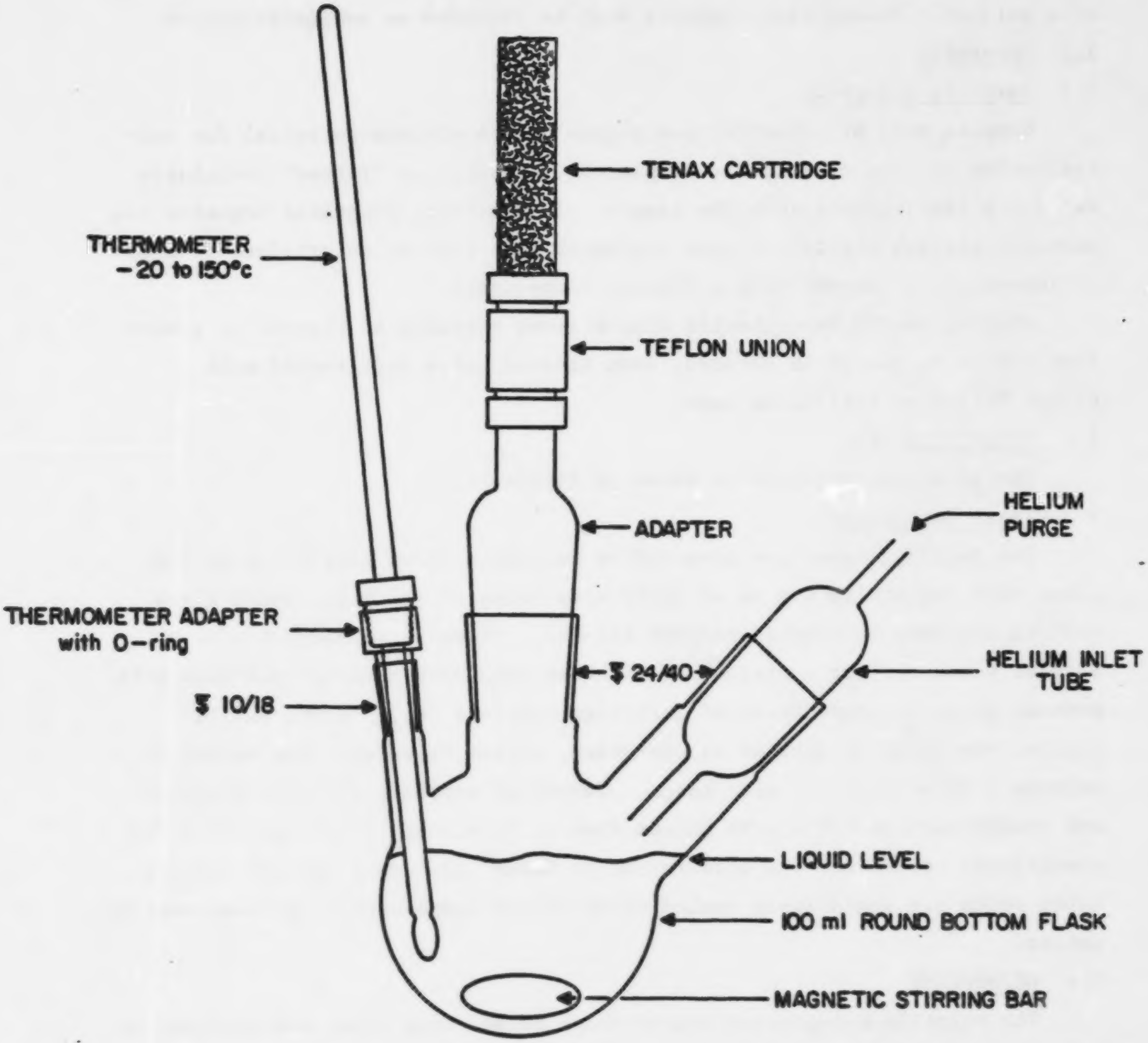
TENAX CARTRIDGE
THERMOMETER
-20 to 150°c
TEFLON UNION
ADAPTER
HELIUM PURGE
THERMOMETER ADAPTER with O-ring
₮ 24/40
HELIUM INLET TUBE
₮ 10/18
LIQUID LEVEL
100 ml ROUND BOTTOM FLASK
MAGNETIC STIRRING BAR

**Figure E-1. Headspace purge apparatus for blood, urine, and tissue samples.**

E5). The operating conditions for the thermal desorption unit and the analysis of Tenax GC cartridges are given in Table E-2.

## 6.0 Procedure

### 6.1 Collection of Samples

It is anticipated that tissue samples collected from cadavers or surgery will be obtained from a pathologist. Personnel from RTI will work with pathologists advising them of proper sample handling procedures. To be of use for purgeable halogeanted organics a tissue sample must be collected a short time following death and immediately frozen in a cleaned glass container with as small a "headspace" as possible. Any handling or storage in contact with polymeric materials represents potential contamination.

### 6.2 Purge of Volatile Organics

An aliquot (5 g) of frozen tissue is cut from a larger mass, sectioned and transferred to the purge flask in Figure E-1. Purged, distilled water is added to the sample to dilute it to about 50 ml; and the mixture macerated in an ice bath using a Virtis tissue homogenizer. The purging apparatus is immediately assembled, stirring started, the temperature raised to 50°C, and the helium flow started at 25 ml/min. After 30 min, the purge is terminated, the apparatus disassembled, and the Tenax cartridge stored in a Kimax culture tube with calcium sulfate desiccant in a freezer until it is ready for analysis.

### 6.3 Analysis of Sample Purged on Cartridge

The instrumental conditions for the analysis of halogenated hydrocarbons of the sorbent Tenax GC sampling cartridge is shown in Table E-2. The thermal desorption chamber and six-port valve are maintained at 270° and 200°C, respectively. The helium purge gas through the desorption chamber is adjusted to 15-20 ml/min. The nickel capillary trap at the inlet manifold is cooled with liquid nitrogen. In a typical thermal desorption cycle a sampling cartridge is placed in the preheated desorption chamber and helium gas is channeled through the cartridge to purge the vapors into the liquid nitrogen cooled nickel capillary trap. After desorption the six-port valve is rotated and the temperature on the capillary loop is rapidly raised; the carrier gas then introduces the vapors onto the high resolution GLC column. The glass capillary column is temperature programmed from 20° to 240°C at

Table E-2. INSTRUMENTAL OPERATING CONDITIONS

| | LKB 2091 | Varian MAT CH-7 |
|---|---|---|
| Desorption chamber temperature | 270 | 265 |
| Desorption chamber He flow | 20 ml/min | 10 ml/min |
| Desorption time | 5.0 min | 8.0 min |
| Capillary Trap Temperature during desorption | -196°C | -196°C |
| Temperature of capillary trap during injection onto column | -196°C to 250°C - then held at 190°C | |
| Time of He flow through capillary trap | 12 3/4 min | 12 3/4 min |
| He flow through column [sweep time] | 9.5 min | 5 min |
| Carrier flow | 2.0 ml/min | 2.5 ml/min |
| Capillary column | 100 m SE-30 SCOT | |
| Column temperature | 30°C for 2 min, then 4°/min to 240° | 20 → 240° at 4°/min |
| Scan range | 5-490 dalton | 20 → 500 dalton |
| Scan rate | 1 sec full scale | 1 sec/decade |
| Scan cycle time | 2.4 sec | 4.5 sec |
| Scan mode | parabolic | exponential |
| Trap current | 4A | |
| Filament current | 50 μA | 200 μA |
| Accelerating volatage | 3.5 kV | 2 kV |

4°/min and held at the upper limit for a minimum of 10 min. After all of the components have eluted from the capillary column the analytical column is then cooled to ambient temperature and the next sample is processed (E2).

## 6.4 Quantitation

All data are acquired in the full scan mode. Quantitation of the halogenated compounds of interest is accomplished by utilizing selected ion plots, SIPs, which are plots of the intensity of specific ions (obtained from full scan data) vs. time. Using SIPs of ions characteristic of a given compound in conjunction with retention times permits quantitation of components of overlapping peaks. Two external standards, perfluorobenzene and perfluorotoluene, are added to each Tenax GC cartridge in known quantities just prior to analysis. In order to eliminate the need to construct complete calibration curves for each compound quantitated, the method of relative molar response (RMR) is used. In this method the relationship of the RMR of the unknown to the RMR of the standard is determined as follows:

$$\mathrm{RMR}_{\mathrm{unknown}} = \frac{A_{\mathrm{unk}}/\mathrm{Moles}_{\mathrm{unk}}}{A_{\mathrm{std}}/\mathrm{Moles}_{\mathrm{std}}},$$

$$\mathrm{RMR}_{\mathrm{unk/std}} = \frac{A_{\mathrm{unk}}/g_{\mathrm{unk}}/\mathrm{GMW}_{\mathrm{unk}}}{A_{\mathrm{std}}/g_{\mathrm{std}}/\mathrm{GMW}_{\mathrm{std}}}$$

where A = peak response of a selected ion,

g = number of grams present, and

GMW = gram molecular weight.

Thus, in the sample being analyzed:

$$g_{\mathrm{unknown}} = \frac{(A_{\mathrm{unk}})(\mathrm{GMW}_{\mathrm{unk}})(g_{\mathrm{std}})}{(A_{\mathrm{std}})(\mathrm{GMW}_{\mathrm{std}})(\mathrm{RMR}_{\mathrm{unk/std}})}.$$

The value of an RMR is determined from at least three independent analyses of standards of accurately known concentration prepared using a gas permeation system (E5). The precision of this method has been determined to be generally ±10 percent when replicate sampling cartridges are examined.

## 7.0 Quality Assurance Program

In addition to the validation procedures described above, an ongoing quality assurance program is required to assure the data quality. Quality control procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance procedures monitor the execution of the procedure and check data interpretation and calculations.

## 7.1 Quality Control

### 7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each of the anticipated number of field samples. Blanks consist of 5 g of tissue or 50 ml water in the same type of sampling container as is used in the field. Controls consist of 5 g of tissue or 50 ml water spiked with 100-500 ng of each of the compounds listed in Table E-1. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

### 7.1.2 Procedure of Blanks and Controls

### 7.1.2.1 Tenax GC

The purity of each batch of Tenax GC cartridges is checked by thermal desorption/GC/FID. An "acceptable background" is judgement based upon the backgrounds obtained previously which exhibited no GC/MS artifacts.

### 7.1.2.2 Purge Blanks

With each set of purge samples, a procedural blank is run. This consists of 50 ml of prepurged distilled water which is purged under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion and storage.

### 7.1.2.3 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a Tenax GC cartridge directly loaded with standards is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

## 7.2 Quality Assurance

### 7.2.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

### 7.2.2 Documentation

#### 7.2.2.1 Chain of Custody

From the initial preparations of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

#### 7.2.2.2 Sampling Protocol Sheets

When a sample is collected, a Sampling Protocol Sheet is filled in which contains a discrete sample code which identifies project number, area, site, location, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

#### 7.2.2.3 Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

#### 7.2.2.4 GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data have been produced.

## 8.0 References

E1. Pellizzari, E. D., Development of Method for Carcinogenic Vapor Analysis in Ambient Atmospheres. Publication No. EPA-650/2-74-121, Contract No. 68-02-1228, 148 pp., July, 1974.

E2. Pellizzari, E. D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors. Publication No. EPA-600/2-76-076, Contract No. 68-02-1228, 185 pp., November, 1975.

E3. Pellizzari, E. D., J. E. Bunch, B. H. Carpenter and E. Sawicki, Environ. Sci. Tech., 9, 552 (1975).

E4. Pellizzari, E. D., B. H. Carpenter, J. E. Bunch and E. Sawicki, Environ. Sci. Tech., 9, 556 (1975).

E5. Pellizzari, E. D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors, Publication No. EPA-600/7-77-055, 288 pp., June 1977.

Revised April, 1979.

# APPENDIX F

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN BLOOD

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN BLOOD

## 1.0 Principle of Method

Semi-volatile halogenated hydrocarbons are extracted from blood plasma with organic solvents, dried, and concentrated to an appropriate volume for quantification using gas chromatograph/electron capture detector (GC/ECD). Identifications are confirmed by GC/ECD using a second column and, when sufficiently concentrated, by GC/MS/COMP. Blood samples are optionally subjected to liquid chromatographic cleanup on Florisil if severe interferences are encountered. This procedure was adapted from that of Thompson (F1).

## 2.0 Range and Sensitivity

The sensitivity of response to GC/ECD is a function of the instrument, the compound, and the matrix from which it is extracted. Acceptable recoveries from human plasma at approximately 5 ppb (parts per billion) have been achieved.

## 3.0 Interferences

Interferences in sample analysis and quantification using GC/ECD are manifested in the electron capturing ability of the given contaminant. Blood extracts which have not been cleaned up contain rapidly eluting components which exhibit high electron capturing properties; these interferences can largely be removed by gradient liquid-liquid chromatography on 2% aqueous deactivated Florisil, as discussed below.

## 4.0 Precision and Accuracy

Recovery studies were initiated with a wide variety of model halogenated hydrocarbons (*e.g.*, trifluralin, α-BHC, β-BHC, γ-BHC, 2,4,5-trichlorobiphenyl, heptachlor, aldrin, heptachlor epoxide, endosulfan, *p*,*p*'-DDE and dieldrin). For 2.0 ml aliquots of human plasma spiked with 14 ng of the above halogenated hydrocarbons and equilibrated for 19 hr at 4°C, a mean recovery of 53.1 ± 12.6% was obtained. The recovery data is reproduced in Table F-1.

## Table F-1. RECOVERY STUDIES OF EXTRACTABLE HALOGENATED HYDROCARBONS FROM HUMAN PLASMA

| | | Recovery Plasma (%) | | | |
|---|---|---|---|---|---|
| Compound | ng spiked | Trial One | Trial Two | Trial Three | Mean |
| Trifluralin | 14.8 | 87.6 | 92.3 | 83.3 | 85.7 |
| α-BHC | 14.0 | 61.9 | 56.2 | 57.6 | 58.6 |
| β-BHC | 14.5 | 60.0 | 60.1 | 43.8 | 50.8 |
| γ-BHC | 15.2 | 57.1 | 53.3 | 52.4 | 54.3 |
| 2,4,5-Trichlorobiphenyl | 14.0 | 48.2 | a | a | 48.2 |
| Heptachlor | 13.6 | 50.2 | 42.2 | 51.1 | 47.8 |
| Aldrin | 14.8 | a | 40.3 | 37.2 | 38.8 |
| Heptachlor epoxide | 14.2 | 35.2 | 43.1 | a | 39.2 |
| Endosulfan | 13.1 | 53.2 | 59.1 | 44.3 | 52.2 |
| *p*,*p*'-DDE | 11.6 | 51.4 | a | 48.8 | 50.1 |
| Dieldrin | 15.8 | 58.6 | a | 58.5 | 58.6 |
| Mean ± Std. Dev. | | | | | 53.1 ± 12.6 |

[a] Not quantifiable due to instrumental problems.

## 5.0 Apparatus

### 5.1 Sampling Apparatus

Blood samples are obtained in 10 ml vacutainer tubes "suitable for GC" (Venoject L428, Kimble) containing 15 mg tripotassium salt of EDTA and 20 μg potassium sorbate.

### 5.2 Extraction Apparatus

Glass culture tubes (16 x 125 mm) and caps equipped with Teflon liners, reciprocal shaker (ca. 40 oscillations/minute), 500 ml Kuderna-Danish evaporators (or 10 ml microevaporators) and receiving tubes, three ball Snyder columns, glass bottles and caps equipped with Teflon liners, reactivials®, centrifuge, and 22 mm i.d. chromatography columns. Solvent: pentane, distilled in glass and redistilled prior to use. Reagents: anhydrous sodium sulfate, (extracted with pentane in Soxhlet extractor for 24 hr and stored in an oven at 140°C), 60/100 mesh reagent Florisil.

## 6.0 Procedure

### 6.1 Collection of Samples

Blood is obtained from volunteers by a qualified phlebotomist using vacutainers. Samples are inverted to ensure dispersal of the anticoagulant and are then centrifuged. Plasma is then pipetted off, stored in a shell vial with teflon-lined screw caps and frozen as soon as possible.

### 6.2 Extraction, Cleanup, and Concentration

Exactly 2.0 ml of plasma is transferred to a 16 x 125 mm culture tube. Hexane (7.0 ml) is then pipetted in, the culture tubes capped, and the samples placed on the shaker bath at 40 oscillations/minute for 24 hours. The samples are removed from the bath, 5.0 ml of organic extract is withdrawn and transferred to glass bottles with Teflon liners, and the samples are returned to the shaker bath for 24 hours with 5.0 ml additional fresh hexane. Again, 5.0 ml are withdrawn, and the combined extracts are dried a minimum of 30 minutes over about 0.5 g anhydrous $Na_2SO_4$. The extract is transferred to a 500 ml KD flask (or micro KD), topped with a Synder column, concentrated to ca. 2-4 ml, and cooled to ambient temperature. The sides of the KD are rinsed with about 1.5 ml hexane and the extract is then blown down under nitrogen to about 1 ml, transferred to a reactivial® previously calibrated to a specific volume, and further concentrated.

## 6.3 Instrumental

The detection and quantification of semi-volatile halogenated hydrocarbons is made using a Series 4400 Fisher/Victoreen Gas Chroamtograph equipped with a tritium foil electron capture detector. Separation is effected on a 40 m, 0.38 mm i.d., glass SCOT capillary column coated with 1% SE-30 on 0.32% Tullanox (F2,F3). Maximum efficiency is obtained with a flow rate of 2.5 ml/min of nitrogen gas with makeup nitrogen gas adjusted to a total flow of 25.0 ml/min, column 220°C (isothermal), and detector 285°C.

As a confirmatory column a 190 cm x 0.2 cm i.d. 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W-HP packing is employed. Efficient responses are obtained for flow rates of 18 ml/min at identical column and detector temperatures.

Final confirmation of the identity of the components of sufficiently concentracted extracts (generally greater than 10 ng/µl) can be made using gas chromatography/mass spectrometry/computer (GC/MS/COMP) (Finnigan 3300).

The GC/MS/COMP systems used are a Finnigan 3300 GC/MS/COMP and an LKB 2091 GC/MS equipped with an LKB 2031 data system. Chromatographic conditions for the Finnigan 3300 are 20 m x 0.38 mm i.d., 1% SE-30 SCOT capillary operated isothermally at 235°C and a flow rate of 2.0 ml/min helium. Splitless injection (0.2-0.3 µl) is used, with standard electron impact (70 eV) ionization conditions.

The LKB 2091 is operated using a 18 m 1% SE-30/$BaCO_3$ WCOT capillary column at 240°, isothermal for PCBs and a 40 m x 0.38 mm i.d. 1% SE-30 SCOT capillary column at 230° isothermal for the pesticides. In both cases, the column flow rate is 2 ml/min with 20 ml/min split off at the injector. The mass spectrometer is operated under standard electron impact conditions.

## 6.4 Qualitative and Quantitative Analysis

### 6.4.1 Qualitative Analysis

Alternate single injection of extracts and standard solutions is the routine procedure for processing samples. If the retention time of a given component of an extract suggests the presence of a standard compound, a repetitive injection is then made. Tentative identification is made if the deviation between the two respective means is no greater than three percent. A similar criterion is then applied to the retention times of both extract and standard component upon a second confirmatory column. Qualitative identification of a component is made if both criteria are satisfied.

### 6.4.2 Quantitative Analysis

A mean linear response range of 5-160 pg/µl has been established for the compounds trifluralin and γ-BHC on a 1% SE-30/0.32% Tullanox 40 m, 0.38 mm i.d. SCOT capillary column installed in a Series 4400 Fisher/Victoreen Gas Chromatograph. Quantification of a given component is made by a comparison of the means of recorder trace areas of two extract and two standard solutions within this linear response range. The precision of the concentration of a given component is normally less than ten percent of the mean concentrations and is obtained by propagation of the standard deviations of the responses of both the extract and standard solutions. The effective concentration multiplied by the volume of extract results in the total amount of extracted material.

The areas employed in the quantification of blood extracts must be normalized to compensate for the volume of extract that is not analyzed. The removal of the first 5.0 ml aliquot from the 7.0 ml extract leaves 2/7 of the extracted material behind. Since the partition coefficients for semi-volatiles in human plasma are large, one approximates that the second extraction contributes more as a dilution gradient than as an extracting solvent. Under this approximation, 5/7 of the original 2/7 remaining in the organic extract would be obtained from the second aliquot. One would then have $5/7 + (2/7)\cdot(5/7) = 45/49$ of the total initial extract to analyze, and the area responses for the extracts would necessarily require normalization by $49/45 = 1.089$ prior to comparison to standard solutions. This methodology was initiated due to the formation of complex emulsions between the plasma and organic solvent which prevented the easy removal of all of the organic extract. This procedure assumes an infinite partition coefficient and that the second extraction does not influence the total recovery.

If the extracts are colored, the presence of lipids may interfere with either analysis or concentration of the extracts due to precipitation. In this case, the sample should be transferred to a 22 mm i.d. column containing 1.6 g of 2% aqueous-deactivated Florisil and eluted with 10 ml each of hexane, 5% $MeCl_2$/hex, 10% $MeCl_2$/hex, 15% $MeCl_2$/hex, 20% $MeCl_2$/hex, 30%

$MeCl_2$/hex, 50% $MeCl_2$/hex, and $MeCl_2$. The extracts are concentrated and analyzed. Most semi-volatile halogenated hydrocarbons are expected to appear in the first five fractions. This estimate is based upon elution data of pesticides on Florisil (F4) and has not been subjected to full experimental verification.

### 6.4.3 GC/MS/COMP Confirmation

The chromatography conditions are similar to those used for GC/ECD. The samples for this study are to be screened by GC/ECD and confirmed (if sufficiently concentrated) by GC/MS/COMP. Therefore, the retention times of the two techniques must be similar. GC/ECD must operate isothermally, so the GC/MS/COMP conditions reflect this restriction.

The Finnigan 3300 and the LKB 2091 systems may be operated in both the full scan and selected ion monitoring (SIM) modes. In the full scan mode, full spectra are collected. Spectra or mass fragmentograms (single ion plots) may be plotted for interpretation. In the SIM mode, only a small number (up to 9 for the Finnigan 3300 and up to 16 for the LKB 2091) of ions are monitored. Full spectra are not collected. The advantage of this method is that the detector spends more time "looking" at the selected ion and therefore better (generally 10-50 times) sensitivity is obtained.

To determine the limits of detection, standard solutions of selected pesticides and PCB isomers have been analyzed on the Finnigan 3300 and LKB 2091. In the full scan mode, the limit of detection was the amount of compound required for an interpretable spectrum. In the SIM mode, the limit of detection was the amount of compound required to yield a peak 2-4 times the noise level.

The estimated limits of detection for the Finnigan 3300 and LKB 2091 are presented in Table F-2.

Quantitation using GC/MS/COMP is achieved by comparing the computer-calculated integrated area of the unknown with the integrated response for a known amount of standard. To compensate for differences in ionization cross-section, the relative molar response of authentic compounds is obtained.

The calculation of the relative molar response (RMR) factor allows the estimation of the levels of sample components without establishing a calibration curve. The RMR is calculated as the integrated peak area of a known

Table F-2. ESTIMATED LIMITS OF DETECTION FOR EXTRACTABLE HALOGENATED ORGANICS ANALYSIS[a]

| | LKB 2091[b] | | | Finnigan 3300[a] | | |
|---|---|---|---|---|---|---|
| | Full Scan | SIM | | Full Scan | SIM | |
| Compound | ng/μl | m/z | ng/μl | ng/μl | m/z | ng/μl |
| trifluralin | 12 | 264 | 0.4 | 5-10 | 264 | <0.5 |
| atrazine | >12<20 | 200 | 0.4 | <50 | 200 | 5-10 |
| γ-BHC (lindane) | >12<20 | 181 | 0.10-0.4 | 5-10 | 181 | 1 |
| heptachlor | 12 | 272 | 0.10-0.4 | 10-20 | 272 | 1-1.5 |
| chlordane | ∿30[c] | 375 | 5 | 25-50 | 375 | 5-10 |
| *p*,*p*'-DDE | 12 | 246 | >0.3 | 5-10 | 246 | 0.5-1 |
| 2-chlorobiphenyl | ∿1 | 188 | 0.004 | ∿2.5 | 188 | ∿0.025 |
| hexachlorobiphenyl | <1 | 360 | ∿0.016 | 25-50 | 360 | ∿0.15 |
| decachlorobiphenyl | 12 | 498 | 0.42 | 150 | 498 | ∿0.3 |

[a] See text for conditions.

[b] 15:1 split at injection, only 1/15 of injection is on column.

[c] 0.2 μl injected with no split.

amount of the compound, $A^{\circ}_{unk}$, with respect to the integrated peak area of a known standard, $A^{\circ}_{std}$ (in this case $d_{10}$-pyrene), according to the equation

$$R = \frac{A^{\circ}{}_{unk}/\text{moles}_{unk}}{A^{\circ}{}_{std}/\text{moles}_{std}} = \frac{(A^{\circ}{}_{unk})\ (mw_{unk})\ (g_{std})}{(A^{\circ}{}_{std})\ (mw_{std})\ (g_{unk})} \qquad \text{(Eq. 1)}$$

From this calculated value, the concentration of an identified compound in a sample is calculated by rearranging Equation 1 to give

$$g_{unk} = \frac{(A_{unk})\ (mw_{unk})\ (g_{std})}{(A_{std})\ (mw_{std})\ (RMR)} \qquad \text{(Eq. 2)}$$

The use of RMR for quantitation by GC/MS has been successful in repeated applications to similar research problems.

The RMRs for the compounds were calculated from the numerical integrations of peaks observed in the appropriate MID channel. Typical RMRs listed in Table F-3 and F-4 are mean values of three injections of each of three replicate standard mixtures.

The RMRs given here are to be regarded as typical values. Not only must they be determined for each instrument, but day-to-day variations are sometimes large enough to require daily calibration.

## 7.0 Quality Assurance Program

In addition to the validation procedures described above, an on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

## 7.1 Quality Control

### 7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each of the anticipated number of field samples. Blanks consist of 10 ml of water in the same type of sampling container as is used in the field. Controls consist of 10 ml of plasma spiked at 10-15 ng with the compounds listed in Table F-5. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and

## Table F-3. RMRs FOR PCBs AND PESTICIDES OF INTEREST TO THIS PROGRAM[a]

| Compound | Concentration | Ion | RMR |
|---|---|---|---|
| 2-chlorobiphenyl | 104 ng/µl | 188 | elutes with solvent and was two scans wide - not determinable |
|  | 3.8 ng/µl |  |  |
| hexachlorobiphenyl | 570 ng/µl | 360 | .38 ± 3% |
|  | 10.4 ng/µl |  | .35 ± 10% |
| decachlorobiphenyl | 1156 ng/µl | 498 | .14 ± 7% |
|  | 8.4 ng/µl |  | not determinable |
| trifluralin | 100 ng/µl | 264 | 1.32 ± 20% |
| atrazine | 100 ng/µl | 200 | .74 ± 7% |
|  |  | 202 | .25 ± 8% |
| lindane | 100 ng/µl | 181 | .74 ± 9% |
|  |  | 183 | .62 ± 12% |
| heptachlor | 100 ng/µl | 272 | .74 ± 6% |
| *p*,*p*'-DDE | 100 ng/µl | 246 | .45 ± 6% |
| chlordane (peak 1) | 100 ng/µl | 373 | .71 ± 5% |
|  |  | 375 | .65 ± 5% |
| chlordane (peak 2) | 100 ng/µl | 373 | .051 ± 6% |
|  |  | 375 | .045 ± 13% |

[a] Standard is $d_{10}$-pyrene ($m/z$ = 212).

## Table F-4. RMR FACTORS FOR STANDARD PCB SOLUTIONS, SELECTED ION MONITORING MODE

| Standard | RMR m/z 188 2-Chlorobiphenyl | | RMR m/z 358 Hexachlorobiphenyl | | RMR m/z 498 Decachlorobiphenyl | |
|---|---|---|---|---|---|---|
| I PCB-STD-20 | 0.60 | | 0.257 | | 0.341 | |
| II PCB-STD-2 | 0.620<br>0.811<br>0.466<br>0.643 | 0.640 ± .171 | 0.291<br>0.334<br>0.319<br>0.321 | 0.325 ± .009 | 0.430<br>0.474<br>0.462<br>0.431 | 0.456 ± .018 |
| III PCB-STD-0.2 | 0.566<br>0.840<br>0.637<br>0.597<br>0.705 | 0.699 +.171 | 0.366<br>0.293<br>0.301<br>0.239<br>0.273 | 0.294 ± .072 | 0.372<br>0.361<br>0.373<br>0.303<br>0.394 | 0.361 ± .033 |
| IV PCB-STD-0.04 | 1.020<br>0.692<br>0.576 | 0.763 ± .257 | 0.320<br>0.528<br>0.528 | 0.459 ± .072 | 0.287<br>0.543<br>0.372 | 0.401 ± .142 |

[a] Standard is $d_{10}$-pyrene (m/z = 212).

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## Table F-5. SEMI-VOLATILE HALOGENATED HYDROCARBONS IN METHANOL SPIKING SOLUTION

| Compound | Amt. spiked, ng |
|---|---|
| 4-Chlorobiphenyl | 13.2 |
| Trifluralin | 14.8 |
| α-BHC | 14.0 |
| β-BHC | 14.5 |
| γ-BHC | 15.2 |
| 4,4'-Dichlorobiphenyl | 15.8 |
| 2,4,5-Trichlorobiphenyl | 14.0 |
| Heptachlor | 13.6 |
| Aldrin | 14.8 |
| Heptachlor epoxide | 14.2 |
| Endosulfan | 13.1 |
| Dieldrin | 15.8 |
| p,p'-DDE | 11.6 |
| p,p'-DDT | 12.5 |
| Endrin | 11.5 |

analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

### 7.1.2 Procedural Blanks and Controls

#### 7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 10 ml of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

#### 7.1.2.2 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

The Finnigan 3300 GC/MS is a quadrupole mass spectrometer which requires frequent tuning. Daily tuning is achieved using FC-43 and decafluorotriphenylphosphine (DFTPP).

## 7.2 Quality Assurance

### 7.2.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

### 7.2.2 Documentation

#### 7.2.2.1 Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

#### 7.2.2.2 Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

#### 7.2.2.3 Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical procotol.

#### 7.2.2.4 GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

## 8.0 References

F1. Thompson, J. F., Analysis of Pesticide Residues in Human and Environmental Samples, A Compilation of Methods Selected for Use in Pesticide Monitoring Programs, Environ. Toxicol. Div., Health Effects Research Lab., USEPA, RTP, NC, December, 1974.

F2. Hines, J. W., R. Shapiro, E. Pellizzari and A. Schwartz, HRC and CC, submitted for publication (1978).

F3. Hines, J. R., R. Shapiro, A. Schwartz, and E. D. Pellizzari, HRC and CC, submitted for publication (1978).

F4. Sherma, J., Manual of Analytical Quality Control for Pesticides and Related Compounds, EPA-600/1-76-017, 1976, Table 7-1.

Revised, April, 1979

# APPENDIX G

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN WATER

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN WATER

## 1.0 Principle of Method

Semi-volatile halogenated hydrocarbons are extracted from water samples with organic solvents, dried, and concentrated to an appropriate volume for quantification using GC/ECD. Results are confirmed by GC/MS/COMP if the sample is sufficiently concentrated. This procedure was adapted from that of Thompson (G1).

## 2.0 Range and Sensitivity

The sensitivity of response to GC/ECD is a function of the instrument, the compound, and the matrix from which it is extracted. Detection limits of approximately 5 ppt (parts per trillion) have been established for water extracts (120 ml aliquots) concentrated to 100 µl.

## 3.0 Interferences

Interferences in sample analysis and quantification using GC/ECD are manifested in the electron capturing ability of the given contaminant. The relative purity of the water matrices (i.e., residential drinking water) minimizes large interferences and largely eliminates the necessity of sample cleanup prior to analysis.

## 4.0 Precision and Accuracy

Recovery studies were initiated with a wide variety of model halogenated hydrocarbons (e.g., trifluralin, α-BHC, β-BHC, γ-BHC, 2,4,5-trichlorobiphenyl, heptachlor, aldrin, heptachlor epoxide, endosulfan, p,p'-DDE and dieldrin). For 100 ml of deionized $H_2O$ spiked and equilibrated for 19 hours at 4°C with about 14 ng of the above halogenated hydrocarbons, a mean recovery of 70.7 ± 14.4% was obtained, while at 500 ng spiking, the recovery was 74.0 ± 21.7%. These data are shown in Table G-1.

## 5.0 Apparatus

### 5.1 Sampling Apparatus

Water samples are taken in either 120 ml or 1.0 l prewashed glass bottles which were baked in an oven at 500°C and sealed with prewashed plastic caps and Teflon liners.

Table G-1. RECOVERY STUDIES OF EXTRACTABLE HALOGENATED HYDROCARBONS FROM WATER

| | Recovery (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Low Fortification[a] | | | High Fortification[b] | | | |
| Compound | Trial One | Trial Two | Average | Trial One | Trial Two | Trial Three | Average |
| Trifluralin | 55.4 | 48.1 | 51.8 | 68.3 ± 0.9 | 69.9 ± 4.3 | 126.8 ± 14.0 | 88.3 ± 11.2 |
| α-BHC | 57.8 | 57.9 | 57.8 | 93.2 ± 5.0 | 98.7 ± 6.2 | 82.2 ± 1.2 | 91.4 ± 7.7 |
| β-BHC | 54.7 | 65.1 | 65.1 | - | - | - | - |
| γ-BHC | 65.6 | 70.6 | 68.1 | - | - | - | - |
| 2,4,5-Trichlorobiphenyl | 62.4 | 73.1 | 67.8 | - | - | - | - |
| Heptachlor | 99.1 | 93.6 | 96.4 | 71.0 ± 6.0 | 66.4 ± 4.3 | 83.8 ± 2.0 | 73.7 ± 8.0 |
| Aldrin | 58.3 | 47.8 | 53.0 | - | - | - | - |
| Heptachlor epoxide | 87.4 | 76.1 | 81.8 | - | - | - | - |
| Endosulfan | 78.7 | 76.1 | 78.7 | 81.1 ± 1.4[d] | 103.1 ± 8.7 | 90.9 ± 7.9 | 91.7 ± 11.2 |
| p,p'-DDE | 73.8 | 80.3 | 77.0 | - | - | - | - |
| Dieldrin | 82.0 | 88.6 | 85.3 | 57.5 ± 4.7 | 67.9 ± 8.7 | 63.5 ± 4.9 | 63.0 ± 10.7 |
| Mean ± Std. Dev. | | | 70.7 ± 14.4 | | | | 74.0 ± 21.7 |

[a] 100 ml distilled/deionized water fortified with 11-15 ng of model compound in 100 μℓ methanol, extracted according to protocol.

[b] Samples as (a) except fortification level about 500 ng.

[c] Not quantifiable due to instrumental problem.

[d] Only one standard injection.

## 5.2 Extraction Apparatus

Cylindrical separatory funnels (250 ml) equipped with glass or Teflon stoppers and Teflon stopcocks, reciprocal shaker bath (40 oscillations/minute), 500 ml Kuderna-Danish (KD) evaporators and receiving tubes, three ball Snyder columns, reactivials®. Solvents: pentane and methylene chloride, distilled in glass. Solvents are redistilled prior to use. Reagent: anhydrous sodium sulfate.

## 6.0 Procedure

## 6.1 Collection of Samples

Samples (120 ml) are obtained in triplicate from residential homeowners or other appropriate sources. The first sample is collected immediately and is analyzed for purgeable components. The second and third samples are taken after two minutes of continuous, moderate flow. One of these is analyzed for extractables; the other serves as a backup sample for either purgeables, or extractables. Larger samples (1.0 l) are optionally obtained from water and wastewater treatment plants.

## 6.2 Extraction, Cleanup, and Concentration

Samples are transferred to 250 ml glass cylindrical separatory funnels. Fifteen ml of 15% methylene chloride/hexane ($MeCl_2$/hex) is pipetted in the funnel, the stopper fitted, and the funnel vented. Samples are placed on the shaker bath for fifteen minutes at about 40 oscillations/minute. The organic phase is then transferred into prewashed glass bottles with Teflon liners. The extraction procedure is repeated once, as before, and then once with pure hexane (no methylene chloride). Approximately 0.8 g anhydrous $Na_2SO_4$ is added to the organic extract. A minimum of 30 minutes is allowed for drying. Extracts are then transferred to 500 ml Kuderna-Danish (KD) receiving flasks and are topped with Snyder columns. The extracts are then concentrated to approximately 2-4 ml over a steam bath in a well ventilated hood. The extracts and KD's are allowed to come to ambient temperature, and the sides of the flasks are washed down with approximately 1.5 ml hexane. The receiving vials are then removed, and the extracts are blown down with nitrogen to about 1 ml, transferred to a reactivial® previously calibrated to a specific volume, and further concentrated to 100-300 µl.

## 6.3 Instrumental

The detection and quantification of semi-volatile halogenated hydrocarbons is made using a Series 4400 Fisher/Victoreen Gas Chromatograph equipped with a tritium foil electron capture detector. Separation is effected on a 40 m, 0.38 mm i.d., glass SCOT capillary column coated with 1% SE-30 on 0.32% Tullanox (G2,G3). Maximum efficiency is obtained with a flow rate of 2.5 ml/min of nitrogen gas with makeup nitrogen gas adjusted to a total flow of 25.0 ml/min, column 220°C (isothermal), and detector 285°C.

As a confirmatory column a 190 cm x 0.2 cm i.d. 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W-HP packing is employed. Efficient responses are obtained for flow rates of 18 ml/min at identical column and detector temperatures.

Final confirmation of the identity of the components of sufficiently concentrated extracts (generally greater than 10 ng/μl) can be made using GC/MS/COMP.

The GC/MS/COMP systems used are a Finnigan 3300 GC/MS/COMP and an LKB 2091 GC/MS equipped with an LKB 2031 data system. Chromatographic conditions for the Finnigan 3300 are 20 m x 0.38 mm i.d., 1% SE-30 SCOT capillary operated isothermally at 235°C and a flow rate of 2.0 ml/min helium. Splitless injection (0.2-0.3 μl) is used, with standard electron impact (70 eV) ionization conditions.

The LKB 2091 is operated using a 18 m 1% SE-30/$BaCO_3$ WCOT capillary column at 240°, isothermal for PCBs and a 40 m x 0.38 mm i.d. 1% SE-30 SCOT capillary column at 230° isothermal for the pesticides. In both cases, the column flow rate is 2 ml/min with 20 ml/min split off at the injector. The mass spectrometer is operated under standard electron impact conditions.

## 6.4 Qualitative and Quantitative Analysis

### 6.4.1 Qualitative Analysis

Alternate single injections of extracts and standard solutions is the routine procedure for processing samples. If the retention time of a given component of an extract suggests the presence of a standard compound, a repetitive injection is then made. Tentative identification is made if the deviation between the two respective means is no greater than three percent. A similar criterion is then applied to the retention times of both extract and standard component upon a second confirmatory column. Qualitative identification of a component is made if both criteria are satisfied.

### 6.4.2 Quantitative Analysis

A mean linear response range of 5-160 pg/μl has been established for the compounds trifluralin and γ-BHC on a 1% SE-30/0.32% Tullanox 40 m, 0.38 mm i.d. SCOT capillary column installed in a Series 4400 Fisher/Victoreen Gas Chromatograph. Quantification of given component is made by a comparison of the means of recorder trace areas of two extract and two standard solutions within this linear response range. The precision of the concentration of a given component is normally less than ten percent of the mean concentrations and is obtained by propagation of the standard deviations of the responses of both the extract and standard solutions. The effective concentration multiplied by the volume of extract results in the total amount of extracted material.

### 6.4.3 GC/MS/COMP Confirmation

The chromatography conditions are similar to those used for GC/ECD. The samples for this study are to be screened by GC/ECD and confirmed (if sufficiently concentrated) by GC/MS/COMP. Therefore, the retention times of the two techniques must be similar. GC/ECD must operate isothermally, so the GC/MS/COMP conditions reflect this restriction.

The Finnigan 3300 and the LKB 2091 systems may be operated in both the full scan and selective ion monitoring (SIM) modes. In the full scan mode, full spectra are collected. Spectra or mass fragmentograms (single ion plots) may be plotted for interpretation. In the SIM mode, only a small number (up to 9 for the Finnigan 3300 and up to 16 for the LKB 2091) of ions are monitored. Full spectra are not collected. The advantage of this method is that the detector spends more time "looking" at the selected ion and therefore better (generally 10-50 times) sensitivity is obtained.

To determine the limits of detection, standard solutions of selected pesticides and PCB isomers have been analyzed on the Finnigan 3300 and LKB 2091. In the full scan mode, the limit of detection was the amount of compound required for an interpretable spectrum. In the SIM mode, the limit of detection was the amount of compound required to yield a peak 2-4 times the noise level.

The estimated limits of detection for the Finnigan 3300 and LKB 2091 are presented in Table G-2.

Quantitation using GC/MS/COMP is achieved by comparing the computer-calculated integrated area of the unknown with the integrated response for a known amount of standard. To compensate for differences in ionization cross-section, the relative molar response of authentic compounds is obtained.

The calculation of the relative molar response (RMR) factor allows the estimation of the levels of sample components without establishing a calibration curve. The RMR is calculated as the integrated peak area of a known amount of the compound, $A^{o}_{unk}$, with respect to the integrated peak area of a known standard, $A^{o}_{std}$ (in this case $d_{10}$-pyrene), according to the equation

$$R = \frac{A^{o}{}_{unk}/moles_{unk}}{A^{o}{}_{std}/moles_{std}} = \frac{(A^{o}{}_{unk})\ (mw_{unk})\ (g_{std})}{(A^{o}{}_{std})\ (mw_{std})\ (g_{unk})} \qquad \text{(Eq. 1)}$$

From this calculated value, the concentration of an identified compound in a sample is calculated by rearranging Equation 1 to give

$$g_{unk} = \frac{(A_{unk})\ (mw_{unk})\ (g_{std})}{(A_{std})\ (mw_{std})\ (RMR)} \qquad \text{(Eq. 2)}$$

The use of RMR for quantitation by GC/MS has been successful in repeated applications to similar research problems.

The RMRs for the compounds were calculated from the numerical integrations of peaks observed in the appropriate SIM channel. Typical RMRs listed in Table G-3 and G-4 are mean values of three injections of each of three replicate standard mixtures.

The RMRs given here are to be regarded as typical values. Not only must they be determined for each instrument, but day-to-day variations are sometimes large enough to require daily calibration.

## 7.0 Quality Assurance Program

In addition to the validation procedures described above, an on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system

## Table G-2. ESTIMATED LIMITS OF DETECTION FOR EXTRACTABLE HALOGENATED ORGANICS ANALYSIS[a]

| | LKB 2091[b] | | | Finnigan 3300[a] | | |
|---|---|---|---|---|---|---|
| | Full Scan | SIM | | Full Scan | SIM | |
| Compound | ng/µl | m/z | ng/µl | ng/µl | m/z | ng/µl |
| trifluralin | 12 | 264 | 0.4 | 5–10 | 264 | <0.5 |
| atrazine | >12<20 | 200 | 0.4 | <50 | 200 | 5–10 |
| γ-BHC (lindane) | >12<20 | 181 | 0.10–0.4 | 5–10 | 181 | 1 |
| heptachlor | 12 | 272 | 0.10–0.4 | 10–20 | 272 | 1–1.5 |
| chlordane | ∿30[c] | 375 | 5 | 25–50 | 375 | 5–10 |
| *p*,*p*'-DDE | 12 | 246 | >0.3 | 5–10 | 246 | 0.5–1 |
| 2-chlorobiphenyl | ∿1 | 188 | 0.004 | ∿2.5 | 188 | ∿0.025 |
| hexachlorobiphenyl | <1 | 360 | ∿0.016 | 25–50 | 360 | ∿0.15 |
| decachlorobiphenyl | 12 | 498 | 0.42 | 150 | 498 | ∿0.3 |

[a] See text for conditions.

[b] 15:1 split at injection, only 1/15 of injection is on column.

[c] 0.2 µl injected with no split.

## Table G-3. RMRs FOR PCBs AND PESTICIDES OF INTEREST TO THIS PROGRAM[a]

| Compound | Concentration | Ion | RMR |
|---|---|---|---|
| 2-chlorobiphenyl | 104 ng/μl<br>3.8 ng/μl | 188 | elutes with solvent and was two scans wide - not determinable |
| hexachlorobiphenyl | 570 ng/μl<br>10.4 ng/μl | 360 | .38 ± 3%<br>.35 ± 10% |
| decachlorobiphenyl | 1156 ng/μl<br>8.4 ng/μl | 498 | .14 ± 7%<br>not determinable |
| trifluralin | 100 ng/μl | 264 | 1.32 ± 20% |
| atrazine | 100 ng/μl | 200<br>202 | .74 ± 7%<br>.25 ± 8% |
| lindane | 100 ng/μl | 181<br>183 | .74 ± 9%<br>.62 ± 12% |
| heptachlor | 100 ng/μl | 272 | .74 ± 6% |
| *p*,*p*'-DDE | 100 ng/μl | 246 | .45 ± 6% |
| chlordane (peak 1) | 100 ng/μl | 373<br>375 | .71 ± 5%<br>.65 ± 5% |
| chlordane (peak 2) | 100 ng/μl | 373<br>375 | .051 ± 6%<br>.045 ± 13% |

---

[a] Standard is $d_{10}$-pyrene ($m/z$ = 212).

Table G-4. RMR FACTORS FOR STANDARD PCB SOLUTIONS, SELECTED ION MONITORING MODE

| Standard | RMR m/z 188 2-Chlorobiphenyl | | RMR m/z 358 Hexachlorobiphenyl | | RMR m/z 498 Decachlorobiphenyl | |
|---|---|---|---|---|---|---|
| I PCB-STD-20 | 0.60 | | 0.257 | | 0.341 | |
| II PCB-STD-2 | 0.620<br>0.811<br>0.466<br>0.643 | 0.640 ± .171 | 0.291<br>0.334<br>0.319<br>0.321 | 0.325 ± .009 | 0.430<br>0.474<br>0.462<br>0.431 | 0.456 ± .018 |
| III PCB-STD-0.2 | 0.566<br>0.840<br>0.637<br>0.597<br>0.705 | 0.699 +.171 | 0.366<br>0.293<br>0.301<br>0.239<br>0.273 | 0.294 ± .072 | 0.372<br>0.361<br>0.373<br>0.303<br>0.394 | 0.361 ± .033 |
| IV PCB-STD-0.04 | 1.020<br>0.692<br>0.576 | 0.763 ± .257 | 0.320<br>0.528<br>0.528 | 0.459 ± .072 | 0.287<br>0.543<br>0.372 | 0.401 ± .142 |

[a] Standard is $d_{10}$-pyrene ($m/z$ = 212).

of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

## 7.1 Quality Control

### 7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each of the anticipated number of field samples. Blanks consist of 120 ml of prepurged distilled water in the same type of sampling container as is used in the field. Controls consist of 100 ml of water spiked at 10-15 ng with the compounds listed in Table G-5. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

### 7.1.2 Procedural Blanks and Controls

### 7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 100 ml of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

### 7.1.2.2 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

The Finnigan 3300 GC/MS is a quadrupole mass spectrometer which requires frequent tuning. Daily tuning is achieved using FC-43 and decafluorotriphenylphosphine (DFTPP).

Table G-5. SEMI-VOLATILE HALOGENATED HYDROCARBONS IN METHANOL SPIKING SOLUTION

| Compound | Amt. spiked, ng |
|---|---|
| 4-Chlorobiphenyl | 13.2 |
| Trifluralin | 14.8 |
| α-BHC | 14.0 |
| β-BHC | 14.5 |
| γ-BHC | 15.2 |
| 4,4'-Dichlorobiphenyl | 15.8 |
| 2,4,5-Trichlorobiphenyl | 14.0 |
| Heptachlor | 13.6 |
| Aldrin | 14.8 |
| Heptachlor epoxide | 14.2 |
| Endosulfan | 13.1 |
| Dieldrin | 15.8 |
| p,p'-DDE | 11.6 |
| p,p'-DDT | 12.5 |
| Endrin | 11.5 |

## 7.2 Quality Assurance

### 7.2.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

### 7.2.2 Documentation

#### 7.2.2.1 Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

#### 7.2.2.2 Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

#### 7.2.2.3 Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical procotol.

#### 7.2.2.4 GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

## 8.0 References

G1. Thompson, J. F., Analysis of Pesticide Residues in Human and Environmental Samples, A Compilation of Methods Selected for Use in Pesticide Monitoring Programs, Environ. Toxicol. Div., Health Effects Research Lab., USEPA, RTP, NC, December, 1974.

G2. Hines, J. W., R. Shapiro, E. Pellizzari and A. Schwartz, HRC and CC, submitted for publication (1978).

G3. Hines, J. R., R. Shapiro, A. Schwartz, and E. D. Pellizzari, HRC and CC, submitted for publication (1978).

# APPENDIX H

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN TISSUE

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN TISSUE

## 1.0 Principle of Method

Semi-volatile halogenated hydrocarbons are extracted from tissue samples with organic solvents, dried, and concentrated to an appropriate volume for quantification using GC/ECD. Identifications are confirmed by GC/ECD using a second column and, when sufficiently concentrated, by GC/MS/COMP. Samples are optionally subjected to liquid chromatographic cleanup on Florisil to remove lipids if severe interferences are encountered. This procedure was adapted from that of Thompson (H1).

## 2.0 Range and Sensitivity

The sensitivity of response to GC/ECD is a function of the instrument, the compound, and the matrix from which it is extracted. The detection limit for GC/ECD analysis is 1-5 ng/g (ppb), depending on compound and instrumental conditions.

## 3.0 Interferences

Interferences in sample analysis and quantification using GC/ECD are manifested in the electron capturing ability of the given contaminant. Blood extracts which have not been cleaned up contain interferences which can largely be removed by gradient liquid-liquid chromatography on 2% aqueous deactivated Florisil and/or back partitioning with acetonitrile, as discussed below.

## 4.0 Precision and Accuracy

Preliminary recovery results, shown in Table H-1 indicate that recoveries are from 60-90% for several types of tissue. Further recoveries will be determined with individual samples as they are analyzed throughout the program.

## 5.0 Apparatus

### 5.1 Sampling Apparatus

Samples must be collected and stored with a minimum potential for contamination or loss of more volatile components. The primary cause of contamination is from plasticizers (*e.g.*, phthalates) in plastic and rubber. Therefore contact with these materials must be minimized or eliminated.

Table H-1. RECOVERY OF EXTRACTABLE HALOGENATED HYDROCARBONS FROM HUMAN TISSUE EXTRACTS

| Tissue | Percent Extractable Material[a] | Percent Recovery[b] |
|---|---|---|
| Adipose[c] | 84.3 | 76.6 |
| Brain | 4.4 | 69.7 |
| Liver | 1.1 | 85.1 |
| Kidney | 1.1 | 64.5 |
| Spleen | 1.3 | 91.2 |
| Lung | 0.4 | 87.5 |

[a] Also described as "percent fat" in some procedures.

[b] Recovery of 198 ng aldrin added to tissues after maceration and before extraction as an internal standard. Percent recovery determined after all analytical manipulations. Aldrin represents a suitable standard, since it is metabolized to endrin and has not been found in tissues. Mean recovery for adipose tissues = 79.1 ± 10.6%.

[c] Analytical workup includes acetonitrile partitioning to reduce fat content.

Samples should be stored in glass jars with foil-lined or (preferably) teflon-lined screw caps. The bottles must be thoroughly cleaned and oven-treated prior to use.

## 5.2 Extraction Apparatus

Beakers (500-1000 ml), 500 ml Kuderna-Danish evaporators (or 10 ml microevaporators) and receiving tubes, three ball Snyder columns, glass bottles and caps equipped with Teflon liners, reactivials®, centrifuge, and 22 mm i.d. chromatography columns. Solvent: hexane, distilled in glass and redistilled prior to use. Reagents: sea sand, anhydrous sodium sulfate, (extracted with pentane in Soxhlet extractor for 24 hr and stored in an oven at 140°C), 60/100 mesh reagent Florisil.

## 6.0 Procedure

## 6.1 Collection of Samples

It is anticipated that tissue samples collected from cadavers or surgery will be obtained from a pathologist. Personnel from RTI will work with pathologists advising them of proper sample handling procedures. To be of use for extractable halogenated organics a tissue sample must be collected a short time following death and immediately frozen in a cleaned glass container with as small a "headspace" as possible. Any handling or storage in contact with polymeric materials represents potential contamination.

## 6.2 Extraction, Cleanup, and Concentration

Tissue fractions are analyzed for extractable halogenated organics using a modified procedure by Thompson (H1). Approximately five grams of each tissue is ground in a large glass beaker with acid-washed sea sand and sodium sulfate (both cleaned up prior to use by extraction in a Soxhlet extractor with hexane) using a glass rod until a dry, granular mass is obtained. Aldrin, 198 ng in 100 μl hexane, is added to the sample as an internal standard. Each tissue is then extracted, with vigorous grinding, with three 50 ml aliquots of hexane for approximately 5 min each. The extracts are then filtered, concentrated in a Kuderna-Danish (KD) apparatus to approximately 10 ml, blown down under nitrogen to dryness at room temperature and weighed to obtain a value for the percent extractable material ("percent fat").

The high lipid content of adipose tissue extract necessitates partitioning of the extract into acetonitrile and back-partitioning of the halogenated hydrocarbons into hexane. Approximately 2.5 grams of fat extract is dissolved in 12 ml hexane and extracted two min each with four 30 ml aliquots of redistilled acetonitrile. The extracts are combined with 250 ml 2% aqueous sodium chloride and back-extracted with four 30 ml aliquots of hexane. The extracts are combined, dried over $Na_2SO_4$, concentrated in a KD to about 5 ml, blown down under nitrogen to approximately 2 ml, and subjected to Florisil cleanup as described below.

Florisil (60/100 mesh, activated at 130°C overnight) columns (2.2 cm x 10 cm) with glass frits or glass wool plugs are packed in hexane solvent. Each tissue extract is transferred to the surface of the column in approximately 2 ml of solvent, and the column walls are washed with approximately 4 ml of hexane. The halogenated hydrocarbons are then eluted with 200 ml each of 6% ether/hexane and 15% ether/hexane respectively. Each fraction is concentrated in a KD apparatus to approximately 5 ml. The 6% eluants are blown down under ambient nitrogen to appropriate volumes and immediately analyzed by GC/ECD.

Previous studies by Thompson suggest the following halogenated hydrocarbons should be found in the 6% eluant: BHC isomers, p,p'-DDE, p,p'-DDT, heptachlor, heptachlor epoxide, mirex, PCB, hexachlorobenzene, and trifluralin (H1). These represent the same general polarity of the halogenated compounds of interest in tissue samples, so this fraction is of primary interest for analysis. Extracts are dried a minimum of 30 minutes over about 0.5 g anhydrous $Na_2SO_4$. The extract is transferred to a 500 ml KD flask (or micro KD), topped with a Snyder column, concentrated to ca. 2-4 ml, and cooled to ambient temperature. The sides of the KD are rinsed with about 1.5 ml hexane and the extract is then blown down under nitrogen to about 1 ml, transferred to a reactivial® previously calibrated to a specific volume, and further concentrated.

## 6.3 Instrumental

The detection and quantification of semi-volatile halogenated hydrocarbons is made using a Series 4400 Fisher/Victoreen Gas Chromatograph equipped with a tritium foil electron capture detector. Separation is effected on a

40 m, 0.38 mm i.d., glass SCOT capillary column coated with 1% SE-30 on 0.32% Tullanox (H2,H3). Maximum efficiency is obtained with a flow rate of 2.5 ml/min of nitrogen gas with makeup nitrogen gas adjusted to a total flow of 25.0 ml/min, column 220°C (isothermal), and detector 285°C.

As a confirmatory column a 190 cm x 0.2 cm i.d. 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W-HP packing is employed. Efficient responses are obtained for flow rates of 18 ml/min at identical column and detector temperatures.

Final confirmation of the identity of the components of sufficiently concentracted extracts (generally greater than 10 ng/μl) can be made using GC/MS/COMP.

The GC/MS/COMP systems used are a Finnigan 3300 GC/MS/COMP and an LKB 2091 GC/MS equipped with an LKB 2031 data system. Chromatographic conditions for the Finnigan 3300 are 20 m x 0.38 mm i.d., 1% SE-30 SCOT capillary operated isothermally at 235°C and a flow rate of 2.0 ml/min helium. Splitless injection (0.2-0.3 μl) is used, with standard electron impact (70 eV) ionization conditions.

The LKB 2091 is operated using a 18 m 1% SE-30/$BaCO_3$ WCOT capillary column at 240°, isothermal for PCBs and a 40 m x 0.38 mm i.d. 1% SE-30 SCOT capillary column at 230° isothermal for the pesticides. In both cases, the column flow rate is 2 ml/min with 20 ml/min split off at the injector. The mass spectrometer is operated under standard electron impact conditions.

## 6.4 Qualitative and Quantitative Analysis

### 6.4.1 Qualitative Analysis

Alternate single injections of extracts and standard solutions is the routine procedure for processing samples. If the retention time of a given component of an extract suggests the presence of a standard compound, a repetitive injection is then made. Tentative identification is made if the deviation between the two respective means is no greater than three percent. A similar criterion is then applied to the retention times of both extract and standard component upon a second, confirmatory, column. Qualitative identification of a component is made if both criteria are satisfied.

### 6.4.2 Quantitative Analysis

A mean linear response range of 5-160 pg/μl has been established for the compounds trifluralin and γ-BHC on a 1% SE-30/0.32% Tullanox 40 m, 0.38 mm i.d. SCOT capillary column installed in a Series 4400 Fisher/Victoreen Gas Chromatograph. Quantification of given component is made by a comparison of the means of recorder trace areas of two extract and two standard solutions within this linear response range. The precision of the concentration of a given component is normally less than ten percent of the mean concentrations and is obtained by propagation of the standard deviations of the responses of both the extract and standard solutions. The effective concentration multiplied by the volume of extract results in the total amount of extracted material.

If the extracts are deep yellow, the presence of lipids may interfere with either analysis or concentration of the extracts due to precipitation. In this case, the sample should be transferred to a 22 mm i.d. column containing 1.6 g of 2% aqueous-deactivated Florisil and eluted with 10 ml each of hexane, 5% $MeCl_2$/hex, 10% $MeCl_2$/hex, 15% $MeCl_2$/hex, 20% $MeCl_2$/hex, 30% $MeCl_2$/hex, 50% $MeCl_2$/hex, and $MeCl_2$. The extracts are concentrated and analyzed. Most semi-volatile halogenated hydrocarbons are expected to appear in the first five fractions. This estimate is based upon elution data of pesticides on Florisil (H4) and has not been subjected to full experimental verification.

### 6.4.3 GC/MS/COMP Confirmation

The chromatography conditions are similar to those used for GC/ECD. The samples for this study are to be screened by GC/ECD and confirmed (if sufficiently concentrated) by GC/MS/COMP. Therefore, the retention times of the two techniques must be similar. GC/ECD must operate isothermally, so the GC/MS/COMP conditions reflect this restriction.

The Finnigan 3300 and the LKB 2091 systems may be operated in both the full scan and selective ion monitoring (SIM) modes. In the full scan mode, full spectra are collected. Spectra or mass fragmentograms (single ion plots) may be plotted for interpretation. In the SIM mode, only a small number (up to 9 for the Finnigan 3300 and up to 16 for the LKB 2091) of ions are monitored. Full spectra are not collected. The advantage of this method is that the detector spends more time "looking" at the selected ion and therefore better (generally 10-50 times) sensitivity is obtained.

To determine the limits of detection, standard solutions of selected pesticides and PCB isomers have been analyzed on the Finnigan 3300 and LKB 2091. In the full scan mode, the limit of detection was the amount of compound required for an interpretable spectrum. In the SIM mode, the limit of detection was the amount of compound required to yield a peak 2-4 times the noise level.

The estimated limits of detection for the Finnigan 3300 and LKB 2091 are presented in Table H-2.

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$$R = \frac{A^{\circ}_{unk}/\text{moles}_{unk}}{A^{\circ}_{std}/\text{moles}_{std}} = \frac{(A^{\circ}_{unk})\,(mw_{unk})\,(g_{std})}{(A^{\circ}_{std})\,(mw_{std})\,(g_{unk})} \qquad \text{(Eq. 1)}$$

From this calculated value, the concentration of an identified compound in a sample is calculated by rearranging Equation 1 to give

$$g_{unk} = \frac{(A_{unk})\,(mw_{unk})\,(g_{std})}{(A_{std})\,(mw_{std})\,(RMR)} \qquad \text{(Eq. 2)}$$

The use of RMR for quantitation by GC/MS has been successful in repeated applications to similar research problems.

The RMRs for the compounds were calculated from the numerical integrations of peaks observed in the appropriate SIM channel. Typical RMRs listed in Table H-3 and H-4 are mean values of three injections of each of three replicate standard mixtures.

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| | LKB 2091[b] | | | Finnigan 3300[a] | | |
|---|---|---|---|---|---|---|
| | Full Scan | SIM | | Full Scan | SIM | |
| Compound | ng/µl | m/z | ng/µl | ng/µl | m/z | ng/µl |
| trifluralin | 12 | 264 | 0.4 | 5-10 | 264 | <0.5 |
| atrazine | >12<20 | 200 | 0.4 | <50 | 200 | 5-10 |
| γ-BHC (lindane) | >12<20 | 181 | 0.10-0.4 | 5-10 | 181 | 1 |
| heptachlor | 12 | 272 | 0.10-0.4 | 10-20 | 272 | 1-1.5 |
| chlordane | ∿30[c] | 375 | 5 | 25-50 | 375 | 5-10 |
| *p,p'*-DDE | 12 | 246 | >0.3 | 5-10 | 246 | 0.5-1 |
| 2-chlorobiphenyl | ∿1 | 188 | 0.004 | ∿2.5 | 188 | ∿0.025 |
| hexachlorobiphenyl | <1 | 360 | ∿0.016 | 25-50 | 360 | ∿0.15 |
| decachlorobiphenyl | 12 | 498 | 0.42 | 150 | 498 | ∿0.3 |

[a] See text for conditions.

[b] 15:1 split at injection, only 1/15 of injection is on column.

[c] 0.2 µl injected with no split.

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[a] Standard is $d_{10}$-pyrene ($m/z$ = 212).

Table H-4. RMR FACTORS FOR STANDARD PCB SOLUTIONS, SELECTED ION MONITORING MODE

| Standard | RMR m/z 188 2-Chlorobiphenyl | | RMR m/z 358 Hexachlorobiphenyl | | RMR m/z 498 Decachlorobiphenyl | |
|---|---|---|---|---|---|---|
| I PCB-STD-20 | 0.60 | | 0.257 | | 0.341 | |
| II PCB-STD-2 | 0.620 | 0.640 ± .171 | 0.291 | 0.325 ± .009 | 0.430 | 0.456 ± .018 |
| | 0.811 | | 0.334 | | 0.474 | |
| | 0.466 | | 0.319 | | 0.462 | |
| | 0.643 | | 0.321 | | 0.431 | |
| III PCB-STD-0.2 | 0.566 | 0.699 +.171 | 0.366 | 0.294 ± .072 | 0.372 | 0.361 ± .033 |
| | 0.840 | | 0.293 | | 0.361 | |
| | 0.637 | | 0.301 | | 0.373 | |
| | 0.597 | | 0.239 | | 0.303 | |
| | 0.705 | | 0.273 | | 0.394 | |
| IV PCB-STD-0.04 | 1.020 | 0.763 ± .257 | 0.320 | 0.459 ± .072 | 0.287 | 0.401 ± .142 |
| | 0.692 | | 0.528 | | 0.543 | |
| | 0.576 | | 0.528 | | 0.372 | |

[a] Standard is $d_{10}$-pyrene ($m/z$ = 212).

The RMRs given here are to be regarded as typical values. Not only must they be determined for each instrument, but day-to-day variations are sometimes large enough to require daily calibration.

## 7.0 Quality Assurance Program

In addition to the validation procedures described above, an on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

## 7.1 Quality Control

### 7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each of the anticipated number of field samples. Blanks consist of 50 ml of distilled water in the same type of sampling container as is used in the field. Controls consist of 50 ml of plasma spiked at 10-15 ng with the compounds listed in Table H-5. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

### 7.1.2 Procedural Blanks and Controls

### 7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 5 ml of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

### 7.1.2.2 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve

## Table H-5. SEMI-VOLATILE HALOGENATED HYDROCARBONS IN METHANOL SPIKING SOLUTION

| Compound | Amt. spiked, ng |
|---|---|
| 4-Chlorobiphenyl | 13.2 |
| Trifluralin | 14.8 |
| α-BHC | 14.0 |
| β-BHC | 14.5 |
| γ-BHC | 15.2 |
| 4,4'-Dichlorobiphenyl | 15.8 |
| 2,4,5-Trichlorobiphenyl | 14.0 |
| Heptachlor | 13.6 |
| Aldrin | 14.8 |
| Heptachlor epoxide | 14.2 |
| Endosulfan | 13.1 |
| Dieldrin | 15.8 |
| p,p'-DDE | 11.6 |
| p,p'-DDT | 12.5 |
| Endrin | 11.5 |

as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

The Finnigan 3300 GC/MS is a quadrupole mass spectrometer which requires frequent tuning. Daily tuning is achieved using FC-43 and decafluorotriphenylphosphine (DFTPP).

## 7.2 Quality Assurance

### 7.2.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

### 7.2.2 Documentation

#### 7.2.2.1 Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

#### 7.2.2.2 Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

#### 7.2.2.3 Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical procotol.

#### 7.2.2.4 GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

## 8.0 References

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H4. Sherma, J., Manual of Analytical Quality Control for Pesticides and Related Compounds, EPA-600/1-76-017, 1976, Table 7-1.

Revised, April, 1979

# APPENDIX I

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN SOIL AND SEDIMENT

## ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN SOIL AND SEDIMENT

### 1.0 Principle of Method

Semi-volatile halogenated hydrocarbons are extracted from soil and sediment samples with organic solvents, dried, and concentrated to an appropriate volume for quantification using GC/ECD. Results are confirmed by GC/MS/COMP. This procedure is adapted from that described for PCBs, PCNs and pesticides (I1,I2).

### 2.0 Range and Sensitivity

The sensitivity of response to GC/ECD is a function of the instrument, the compound, and the matrix from which it is extracted. Detection limits of approximately 10 pg/g (ppt) are expected for soil extracts (50 g aliquots) concentrated to 100 µl.

### 3.0 Interferences

Interferences in sample analysis and quantification using GC/ECD are manifested in the electron capturing ability of the given contaminant. Any interferences will be removed using silica gel column chromatography (I1,I2).

### 4.0 Precision and Accuracy

Recoveries from soil on a related project (I3) ranged from 52-162%. Precision and accuracy will be determined for this study using the quality control samples during field sampling.

### 5.0 Apparatus

### 5.1 Sampling Apparatus

A garden bulb planter, trowel, or small shovel.

### 5.2 Extraction Apparatus

Cylindrical separatory funnels (250 ml) equipped with glass or Teflon stoppers and Teflon stopcocks, reciprocal shaker (ca. 40 oscillations/minute), 500 ml Kuderna-Danish evaporators and receiving tubes, three ball Snyder columns, 1.0 l glass bottles and caps equipped with Teflon liners, precleaned glass wool reactivials®. Solvents: pentane and methylene chloride, distilled in glass and redistilled prior to use. Reagents: anhydrous sodium sulfate.

## 6.0 Procedure

### 6.1 Collection of Samples

Soil samples are collected by taking cores with a garden bulb planter. The cores are placed in one liter wide mouth jars, sealed with foil lined caps and taped shut. The sample is labeled and its location and other pertinent data recorded on a protocol sheet.

### 6.2 Extraction, Cleanup, and Concentration

Composite 1/2 of top 2.5 cm of soil core and place in wide mouth jar with other samples to be composited and cap with foil-lined cap. Agitate vigorously to produce a homogeneous sample. A 50 g portion of the composite is then extracted in a 1.0 l wide mouth jar with 50 ml of diether ether (shake for 30 minutes). Decant the ether extract through glass wool into a Kuderna-Danish (K-D) apparatus and combine with subsequent extracts.

The soil residue is then treated with acetone (40 ml) and shaken for 20 minutes. Toluene (80 ml) is added and shaken for 10 minutes. The extract is decanted through a glass wool plug into a 500 ml round bottom flask. The above acetone-toluene extraction is repeated a total of 2 times and the extracts concentrated by heating the round bottom flask with a 3 ball Synder column attached. Final concentration (100 μl) is achieved under a stream of dry nitrogen. All procedures are conducted under minimum light and samples are stored in the dark at 5°C.

### 6.3 Instrumental

The detection and quantification of semi-volatile halogenated hydrocarbons is made using a Series 4400 Fisher/Victoreen Gas Chroamtograph equipped with a tritium foil electron capture detector. Separation is achieved on a 40 m, 0.38 mm i.d., glass SCOT capillary column coated with 1% SE-30 on 0.32% Tullanox (I4,I5). Maximum efficiency is obtained with a flow rate of 2.5 ml/min of nitrogen gas with makeup nitrogen gas adjusted to a total flow of 25.0 ml/min, column 220°C (isothermal), and detector 285°C.

As a confirmatory column a 190 cm x 0.2 cm i.d. 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W-HP packing is employed. Efficient responses are obtained for flow rates of 18 ml/min at identical column and detector temperatures.

Final confirmation of the identity of the components of sufficiently concentracted extracts (generally greater than 10 ng/μl) can be made using GC/MS/COMP.

The GC/MS/COMP systems used are a Finnigan 3300 GC/MS/COMP and an LKB 2091 GC/MS equipped with an LKB 2031 data system. Chromatographic conditions for the Finnigan 3300 are 20 m x 0.38 mm i.d., 1% SE-30 SCOT capillary operated isothermally at 235°C and a flow rate of 2.0 ml/min helium. Splitless injection (0.2-0.3 μl) is used, with standard electron impact (70 eV) ionization conditions.

The LKB 2091 is operated using a 18 m 1% SE-30/$BaCO_3$ WCOT capillary column at 240° isothermal for PCBs and a 40 m x 0.38 mm i.d. 1% SE-30 SCOT capillary column at 230° isothermal for the pesticides. In both cases, the column flow rate is 2 ml/min with 20 ml/min split off at the injector. The mass spectrometer is operated under standard electron impact conditions.

## 6.4 Qualitative and Quantitative Analysis

### 6.4.1 Qualitative Analysis

Alternate single injections of extracts and standard solutions is the routine procedure for processing samples. If the retention time of a given component of an extract suggests the presence of a standard compound, a repetitive injection is then made. Tentative identification is made if the deviation between the two respective means is no greater than three percent. A similar criterion is then applied to the retention times of both extract and standard component upon a second, confirmatory, column. Qualitative identification of a component is made if both criteria are satisfied.

### 6.4.2 Quantitative Analysis

A mean linear response range of 5-160 pg/μl has been established for the compounds trifluralin and γ-BHC on a 1% SE-30/0.32% Tullanox 40 m, 0.38 mm i.d. SCOT capillary column installed in a Series 4400 Fisher/Victoreen Gas Chromatograph. Quantification of a given component is made by a comparison of the means of recorder trace areas of two extract and two standard solutions within this linear response range. The precision of the concentration of a given component is normally less than ten percent of the mean concentrations and is obtained by propagation of the standard deviations of the responses of both the extract and standard solutions. The effective concentration multiplied by the volume of extract results in the total amount of extracted material.

### 6.4.3 GC/MS/COMP Confirmation

The chromatography conditions are similar to those used for GC/ECD. The samples for this study are to be screened by GC/ECD and confirmed (if sufficiently concentrated) by GC/MS/COMP. Therefore, the retention times of the two techniques must be similar. GC/ECD must operate isothermally, so the GC/MS/COMP conditions reflect this restriction.

The Finnigan 3300 and the LKB 2091 systems may be operated in both the full scan and selective ion monitoring (SIM) modes. In the full scan mode, full spectra are collected. Spectra or mass fragmentograms (single ion plots) may be plotted for interpretation. In the SIM mode, only a small number (up to 9 for the Finnigan 3300 and up to 16 for the LKB 2091) of ions are monitored. Full spectra are not collected. The advantage of this method is that the detector spends more time "looking" at the selected ion and therefore better (generally 10-50 times) sensitivity is obtained.

To determine the limits of detection, standard solutions of selected pesticides and PCB isomers have been analyzed on the Finnigan 3300 and LKB 2091. In the full scan mode, the limit of detection was the amount of compound required for an interpretable spectrum. In the SIM mode, the limit of detection was the amount of compound required to yield a peak 2-4 times the noise level.

The estimated limits of detection for the Finnigan 3300 and LKB 2091 are presented in Table I-1.

Quantitation using GC/MS/COMP is achieved by comparing the computer-calculated integrated area of the unknown with the integrated response for a known amount of standard. To compensate for differences in ionization cross-section, the relative molar response of authentic compounds is obtained.

The calculation of the relative molar response (RMR) factor allows the estimation of the levels of sample components without establishing a calibration curve. The RMR is calculated as the integrated peak area of a known amount of the compound, $A^{\circ}_{unk}$, with respect to the integrated peak area of a known standard, $A^{\circ}_{std}$ (in this case $d_{10}$-pyrene), according to the equation

Table I-1. ESTIMATED LIMITS OF DETECTION FOR EXTRACTABLE HALOGENATED ORGANICS ANALYSIS[a]

| | LKB 2091[b] | | | Finnigan 3300[a] | | |
|---|---|---|---|---|---|---|
| | | SIM | | | SIM | |
| Compound | Full Scan ng/μl | m/z | ng/μl | Full Scan ng/μl | m/z | ng/μl |
| trifluralin | 12 | 264 | 0.4 | 5-10 | 264 | <0.5 |
| atrazine | >12<20 | 200 | 0.4 | <50 | 200 | 5-10 |
| γ-BHC (lindane) | >12<20 | 181 | 0.10-0.4 | 5-10 | 181 | 1 |
| heptachlor | 12 | 272 | 0.10-0.4 | 10-20 | 272 | 1-1.5 |
| chlordane | ∿30[c] | 375 | 5 | 25-50 | 375 | 5-10 |
| p,p'-DDE | 12 | 246 | >0.3 | 5-10 | 246 | 0.5-1 |
| 2-chlorobiphenyl | ∿1 | 188 | 0.004 | ∿2.5 | 188 | ∿0.025 |
| hexachlorobiphenyl | <1 | 360 | ∿0.016 | 25-50 | 360 | ∿0.15 |
| decachlorobiphenyl | 12 | 498 | 0.42 | 150 | 498 | ∿0.3 |

[a] See text for conditions.

[b] 15:1 split at injection, only 1/15 of injection is on column.

[c] 0.2 μl injected with no split.

$$R = \frac{A^{\circ}_{unk}/\text{moles}_{unk}}{A^{\circ}_{std}/\text{moles}_{std}} = \frac{(A^{\circ}_{unk})\ (mw_{unk})\ (g_{std})}{(A^{\circ}_{std})\ (mw_{std})\ (g_{unk})} \qquad \text{(Eq. 1)}$$

From this calculated value, the concentration of an identified compound in a sample is calculated by rearranging Equation 1 to give

$$g_{unk} = \frac{(A_{unk})\ (mw_{unk})\ (g_{std})}{(A_{std})\ (mw_{std})\ (RMR)} \qquad \text{(Eq. 2)}$$

The use of RMR for quantitation by GC/MS has been successful in repeated applications to similar research problems.

The RMRs for the compounds were calculated from the numerical integrations of peaks observed in the appropriate SIM channel. Typical RMRs listed in Tables I-2 and I-3 are mean values of three injections of each of three replicate standard mixtures.

The RMRs given here are to be regarded as typical values. Not only must they be determined for each instrument, but day-to-day variations are sometimes large enough to require daily calibration.

## 7.0 Quality Assurance Program

In addition to the validation procedures described above, an on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

### 7.1 Quality Control

### 7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each of the anticipated number of field samples. Blanks consist of 50 g of soil in the same type of sampling container as is used in the field. Controls consist of 50 g of soil spiked at 10-15 ng with the compounds listed in Table I-4. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table I-2. RMRs FOR PCBs AND PESTICIDES OF INTEREST TO THIS PROGRAM[a]

| Compound | Concentration | Ion | RMR |
|---|---|---|---|
| 2-chlorobiphenyl | 104 ng/μl<br>3.8 ng/μl | 188 | elutes with solvent and was two scans wide - not determinable |
| hexachlorobiphenyl | 570 ng/μl<br>10.4 ng/μl | 360 | .38 ± 3%<br>.35 ± 10% |
| decachlorobiphenyl | 1156 ng/μl<br>8.4 ng/μl | 498 | .14 ± 7%<br>not determinable |
| trifluralin | 100 ng/μl | 264 | 1.32 ± 20% |
| atrazine | 100 ng/μl | 200<br>202 | .74 ± 7%<br>.25 ± 8% |
| lindane | 100 ng/μl | 181<br>183 | .74 ± 9%<br>.62 ± 12% |
| heptachlor | 100 ng/μl | 272 | .74 ± 6% |
| *p,p*'-DDE | 100 ng/μl | 246 | .45 ± 6% |
| chlordane (peak 1) | 100 ng/μl | 373<br>375 | .71 ± 5%<br>.65 ± 5% |
| chlordane (peak 2) | 100 ng/μl | 373<br>375 | .051 ± 6%<br>.045 ± 13% |

[a] Standard is $d_{10}$-pyrene ($m/z$ = 212).

Table I-3. RMR FACTORS FOR STANDARD PCB SOLUTIONS, SELECTED ION MONITORING MODE

| Standard | RMR m/z 188 2-Chlorobiphenyl | | RMR m/z 358 Hexachlorobiphenyl | | RMR m/z 498 Decachlorobiphenyl | |
|---|---|---|---|---|---|---|
| I PCB-STD-20 | 0.60 | | 0.257 | | 0.341 | |
| II PCB-STD-2 | 0.620 | 0.640 ± .171 | 0.291 | 0.325 ± .009 | 0.430 | 0.456 ± .018 |
| | 0.811 | | 0.334 | | 0.474 | |
| | 0.466 | | 0.319 | | 0.462 | |
| | 0.643 | | 0.321 | | 0.431 | |
| III PCB-STD-0.2 | 0.566 | 0.699 +.171 | 0.366 | 0.294 ± .072 | 0.372 | 0.361 ± .033 |
| | 0.840 | | 0.293 | | 0.361 | |
| | 0.637 | | 0.301 | | 0.373 | |
| | 0.597 | | 0.239 | | 0.303 | |
| | 0.705 | | 0.273 | | 0.394 | |
| IV PCB-STD-0.04 | 1.020 | 0.763 ± .257 | 0.320 | 0.459 ± .072 | 0.287 | 0.401 ± .142 |
| | 0.692 | | 0.528 | | 0.543 | |
| | 0.576 | | 0.528 | | 0.372 | |

[a] Standard is $d_{10}$-pyrene ($\underline{m}/\underline{z}$ = 212).

## Table I-4. SEMI-VOLATILE HALOGENATED HYDROCARBONS IN METHANOL SPIKING SOLUTION

| Compound | Amt. spiked, ng |
|---|---|
| 4-Chlorobiphenyl | 13.2 |
| Trifluralin | 14.8 |
| α-BHC | 14.0 |
| β-BHC | 14.5 |
| γ-BHC | 15.2 |
| 4,4'-Dichlorobiphenyl | 15.8 |
| 2,4,5-Trichlorobiphenyl | 14.0 |
| Heptachlor | 13.6 |
| Aldrin | 14.8 |
| Heptachlor epoxide | 14.2 |
| Endosulfan | 13.1 |
| Dieldrin | 15.8 |
| p,p'-DDE | 11.6 |
| p,p'-DDT | 12.5 |
| Endrin | 11.5 |

### 7.1.2 Procedural Blanks and Controls

#### 7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 50 ml of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

#### 7.1.2.2 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

The Finnigan 3300 GC/MS is a quadrupole mass spectrometer which requires frequent tuning. Daily tuning is achieved using FC-43 and decafluorotriphenylphosphine (DFTPP).

## 7.2 Quality Assurance

### 7.2.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

### 7.2.2 Documentation

#### 7.2.2.1 Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

#### 7.2.2.2 Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

#### 7.2.2.3 Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical procotol.

#### 7.2.2.4 GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data is archived, and what hardcopy data has been produced.

## 8.0 References

I1. Goerlitz, D. F. and L. M. Law, J. Assoc. Offic. Anal. Chem., 57, 176 (1974).

I2. Erickson, M. D., R. A. Zweidinger, L. C. Michael and E. D. Pellizzari, Environmental Monitoring Near Industrial Sites: EPA 560/6-77-019 (July 1977), 266 pp.

I3. Pellizzari, E. D., R. A. Zweidinger and M. D. Erickson, Environmental Monitoring Near Industrial Sites: Brominated Chemicals. Part II: Appendix, EPA 560/6-78-002, p. 99.

I4. Hines, J. W., R. Shapiro, E. Pellizzari and A. Schwartz, HRC and CC, submitted for publication (1978).

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## APPENDIX J

## ANALYTICAL PROTOCOL: CARCINOEMBRYONIC ANTIGEN ASSAY

# ANALYTICAL PROTOCOL: CARCINOEMBRYONIC ANTIGEN ASSAY

## 1.0 Principle of the Method

A radioimmunoassay (RIA) based on the indirect CEA-Roche assay is presented. In order to detect the low titres of CEA in plasma a perchloric acid extraction is used to remove interferences. The perchloric acid is dialyzed against a buffer before the RIA is performed. The RIA procedure consists of sequential addition of CEA-antiserum, $I^{125}$ labeled CEA and zirconyl phosphate gel (Z-gel). The Z-gel binds the antiserum-CEA complex (CEA plasma + CEA [$I^{125}$]) and determination of the $I^{125}$ in the Z-gel by gamma scintillation counting can be related to the plasma CEA by calibration standards.

## 2.0 Range and Sensitivity

The indirect assay is sensitive from ~0.5 ng/ml to <20 ng/ml. At higher titres a direct assay may be used (see manufacturers literature).

## 3.0 Interferences

The indirect assay was designed to contend with interferences. Interpretation of the origin of elevated CEA levels is a separate issue.

## 4.0 Precision and Accuracy

The precision and accuracy are to some extent a function of the level since response is non-linear. Typically precision is ±0.5 ng/ml from 0-3 ng/ml and ~±1 ng from 3 to 12 ng/ml.

## 5.0 Apparatus

Gamma Scintillation Spectrometer

2.5 ml Dispenser: available from Roche Diagnostics for dispensing of Z-gel

Mixer: Vortex-type mixer

Centrifuge: Capable of generating at least 1000 x g with horizontal-head centrifuge; do not use angle-head type.

## 6.0 Materials

Vacutainer tubes (Becton, Dickinson and Co. Nos. 4759 or 4727) or equivalent.

CEA-Roche Kit

The CEA-Roche Kit contains the following reagents in excess to assure sufficient material for at least 100 tubes or for approximately 40 patient plasma samples assayed in duplicate by the indirect assay with necessary controls.

This kit supplies:

| Item | Contents | Quantity |
|---|---|---|
| CEA Antiserum | goat antiserum adsorbed with blood group A human plasma and saliva from a blood group A positive secretor diluted in sodium borate buffer containing thimerosal 1:10,000 | One 2.5 ml vial (sterile) |
| CEA Standard | 125 ng of CEA activity/ml in sodium borate buffer containing 10% blood group A human plasma and thimerosal 1:10,000 | One 1.25 ml vial (sterile) |
| $^{125}$I-CEA | approximately 50 ng of activity/ml in sodium borate buffer containing 10% blood group A human plasma and thimerosal 1:10,000 | One 2.5 ml vial (not sterile) |
| Zirconyl Phosphate Gel (Z-gel) | in ammonium acetate (0.1 M acetate) solution and thimerosal 1:10,000, pH 6.25. Do not use Z-gel which has been frozen since the material will not be uniformly dispersed and will settle rapidly | One 250 ml bottle (not sterile) |
| EDTA Buffer | stock solution with bovine serum albumin and sodium azide 0.17% as preservative. NOTE: This is to be diluted 1:10 in distilled or deionized water just prior to use (final pH should be 6.5 ± 0.1). It is important to check quality of water before dilution, since if pH is not 6.5 (±0.1), solution must be discarded. | One 25 ml vial (sterile) |

Other materials required:

| | |
|---|---|
| Test Tubes:<br>Disposable test tubes | glass or plastic test tubes can be used; however, user should be consistent. Do not mix glass and plastic as inconsistency of results might occur; 1.5 x 15 cm or 1.2 x 12 cm test tubes are recommended. If a smaller tube is used, it will be necessary to cap the tubes to avoid spillage during the vortex-mixing step, especially after the addition of Z-gel. |

| | |
|---|---|
| Pipettes:<br>10 µl, 25 µl, 50 µl, 100 µl, 500 µl micropipettes | with an accuracy of ±1% |
| Automatic pipetter | 2.5 ml volume - 2 required for perchloric acid and Z-gel. |
| Dialysis Bags: | available presealed from Roche Diagnostics<br>Pore size 15,000 mw; at least 2 cm diameter and cut to 12" length |

Reagents:

| Item | Approximate Quantity Needed/Kit | Directions For Making |
|---|---|---|
| 12 M Perchloric acid:<br>Use: add cold to prepared plasma for precipitating plasma proteins | 0.25 liter | add 108 ml concentrated (70 to 72%) perchloric acid to 892 ml distilled or deionized water; mix carefully and store at 4°C for not longer than one month |
| Normal Saline Solution (0.85% or 0.9%):<br>Use: to dilute plasma samples | 0.25 liter | add 8.5 or 9 gm NaCl to distilled or deionized water; bring volume up to 1 liter and store at room temperature |
| 0.01 M Acetate Buffer, pH 6.5 (±0.2):<br>Use: in the final step of dialysis | 1.0 liter | add 1 ml of stock ammonium acetate solution to 250 ml deionized water (final concentration 0.01 M ammonium acetate); after at least 3 hours to assure mixing and equilibrium of dialysis, final pH of both samples and dialysate should be 6.5 (±0.2). |
| 0.1 M Acetate Buffer, pH 6.25 (±0.05):<br>Use: final 0.1 M solution is used in the wash step of assay | 0.5 liter | dissolve 2.85 ml glacial acetic acid in approximately 400 ml of distilled or deionized water; add concentrated ammonium hydroxide until pH 6.25 is obtained; add distilled or deionized water to bring volume to 500 ml; store for 6 weeks at room temperature |

| Item | Approximate Quantity Needed/Kit | Directions For Making |
|---|---|---|
| Distilled or deionized water (of not less than 1 x $10^6$ ohms): | 250 ml for each dialysis bag | |

Directions for Making 2.5 M Ammonium Acetate Stock Solution:

Add 142.3 ml concentrated acetic acid to 600 ml distilled or deionized water; gradually add 140.0 ml of concentrated ammonium hydroxide while stirring constantly. Upon cooling, add concentrated ammonium hydroxide until pH is 6.8 to 7.0; add distilled or deionized water to make 1 liter and recheck pH; store in air-tight container at room temperature not longer than six weeks. Observe visually for growth before each use.

Important: 1 ml of this solution diluted 1:250 should be checked to be certain that a final pH of 6.5 ± 0.2 will be obtained in the dialysis bath. If the pH of the 0.01 M acetate falls below 6.5, adjust pH of stock ammonium acetate with concentrated ammonium hydroxide - do not exceed 7.0 pH. If pH of stock solution exceeds 7.0, discard. Do Not adjust with acetic acid.

## 7.0 Procedure

### 7.1 Sample Collection, Storage and Shipping

Blood specimens to be analyzed for presence of carcinoembryonic antigen are collected in 7 ml or 10 ml standard Vacutainer tubes (Becton, Dickinson and Company, catalog numbers 4759 or 4727) or equivalent, containing 15 mg EDTA ($K_3$) and 20 µg potassium sorbate in a 0.1 ml solution. The tubes should be filled to exhaustion of vacuum to ensure proper ratio of anticoagulant to blood. Mix by gentle inversion several times.

The blood must be centrifuged at 1000 x g for 30 minutes within six hours after collection if left at room temperature and within 12 hours if refrigerated at 4°C. The supernate plasma must be immediately withdrawn using a disposable Pasteur pipette. This plasma must be refrigerated at 4°C to 8°C until the time of shipment to the laboratory. If the plasma will not be analyzed within two weeks, the sample should be frozen.

## 7.2 CEA-ROCHE Indirect Assay

When setting up a run of the CEA-ROCHE indirect assay, patient specimen should be processed in duplicate. Each run should be accompanied by four (4) control specimens and five (5) concentrations of standards.

| Example | Number of Tubes |
|---|---|
| 20 patient samples run in duplicate | 40 |
| 4 control specimens run in duplicate | |
| 0 to 2.5 ng/ml | 2 |
| 2.6 to 5.0 ng/ml | 2 |
| 5.1 to 10.0 ng/ml | 2 |
| >10 ng/ml | 2 |
| 5 concentrations of standards run in duplicate | |
| 0 ng of CEA activity | 2 |
| 1.25 ng of CEA activity | 2 |
| 3.125 ng of CEA activity | 2 |
| 6.25 ng of CEA activity | 2 |
| 12.5 ng of CEA activity | 2 |
| Total Number of Tubes Run | 58 |

### 7.2.1 Perchloric Acid Extraction of Plasma

Pipette 0.5 ml aliquots of plasma sample or control specimen (in duplicate) into 1.5 x 15 cm or 1.2 x 12 cm test tubes. Add 2 ml of 0.85% of NaCl solution to each tube and mix contents thoroughly on Vortex-type mixer. Add 2.5 ml of 1.2 M cold perchloric acid (PCA) to each tube to precipitate the protein. This reagent should be pipetted only with an automatic pipetter. It should not be dispensed with a mouth-type pipette.

Immediately after the addition of perchloric acid (PCA), mix the contents for 30 seconds vigorously on a Vortex-type mixer. Any time delay between PCA addition and mixing can result in an inconsistent denaturation process. Allow tubes to stand for 5 minutes before centrifuging. Centrifuge tubes for 20 minutes at 1000 x g. Occasionally a turbid sample may be encountered in the perchloric acid extracts, particularly from patients having liver malfunctions. Samples should be processed in a normal manner. If all samples are turbid, centrifuge for an additional 10 minutes.

After centrifugation, a white pellet of precipitated protein at the bottom of the tube and a supernatant fluid are obtained.

### 7.2.2 Dialysis

Dialysis bags should be separated and allowed to soak in deionized water for a minimum of one hour before use. Dialysis bags held for over 18 hours in deionized water before use should be discarded.

Decant supernatant fluids into prewetted dialysis bags. Squeeze excess water from the inner and outer surfaces of the dialysis bag prior to decanting the extracted sample. Tie dialysis bags using double knots, and label. To prevent floating, avoid trapping air in the dialysis bag before knotting. Air can also retard osmosis while dialyzing. The tagged dialysis bag containing the supernatant fluid is now ready to be placed in the dialysis unit.

Load dialysis bag into the dialysis unit. The bags must be submerged below the top knot throughout the entire dialysis procedure. During dialysis, some device should be used to hold each bag in place or label strings will entangle.

Important: At no time during dialysis should metal be allowed to come in contact with the dialysis water as ions would be added thus affecting the results of the assay.

To perform dialysis step without the automatic dialysis unit, one method may be to use an appropriate size beaker with a magnetic stirrer to assure circulation of water and buffer solution. Routine laboratory methods of dialysis are acceptable as long as the volume of dialysis fluid is 50 times the total volume of extracts within the container (250 ml of water is required per bag for each dialysis step). The water should be circulated during the dialysis. If air is used to agitate the water, it should be filtered to remove oil or solids.

Four complete changes of water are required with a minimum of 3 hours between changes. (Dialysis may be run for longer periods without adverse reactions). Dialysis should be performed with water changes as recommended.

After being dialyzed against deionized water, dialyze bags using 50 volumes of 0.01 M ammonium acetate solution having a pH of 6.5 for a minimum of 3 hours and not more than 24 hours. A suggested method for performing this final dialysis is to make an accurate measurement of the dialysis bath fluid volume. Fill to the appropriate volume with water and add a predetermined quantity of 2.5 M buffer to the bath so that dilution of the concentrated buffer with the bath water will equal 0.01 M. (4 ml of 2.5 M ammonium acetate is required for each 1000 ml of water). If the final pH of the dialysis bath, after addition of the buffer, does not read pH 6.5 ± 0.2, DO NOT adjust the bath with concentrated acid or base since this will only increase the ionic strength above 0.01 M. A dialysis bath that does not give a pH of 6.5 ± 0.2 should be discarded and samples redialyzed with the buffer (stock) readjusted to give a final pH of 6.5 ± 0.2 in the dialysis bath. Any pH adjustments must be made to the 2.5 M stock buffer only. Concentrated ammonium hydroxide may be used to adjust the 2.5 M buffer. Acetic acid cannot be used; if the pH of the 0.01 M buffer is above the desired level, the 2.5 M buffer must be discarded and remade.

If the ionic strength or pH of the dialysis buffer is too high, falsely elevated plasma CEA values will be obtained. Dialysis buffer of too low ionic strength or low pH will result in plasma CEA values which are too low.

Remove dialysis bags from the dialysis unit and cut open. The pH of the final dialysis bath should be checked and a pH check of random samples made after dialysis with a pH meter. The pH of the sample must be 6.5 (±0.2). If samples do not have this pH, they should be redialyzed in the final buffer. The difference between the pH of the dialysate and dialysis bath should not vary more than 0.2 pH units. If the variation is greater the dialysis should be continued.

Transfer entire contents of dialysis bags to 1.5 x 15 cm or 1.2 x 12 cm test tubes for determination of CEA content. A low or high volume in the test tube following transfer from the dialysis bag may be indicative of a leaking dialysis bag. When this is encountered, one should be alert to the possibility of a low value.

NOTE: Assays should be performed the same day the contents are removed from the dialysis bags.

### 7.2.3 RIA Assay of CEA

These extracts are now ready to be assayed for CEA content. In order to determine the CEA content of the plasma extract, it is necessary to prepare a standard inhibition curve utilizing the CEA standard in the

CEA-Roche kit. This curve must be prepared at the same time that each bath of plasma extracts is assayed.

Preparation of Standards:

1. Dilute an aliquot of stock EDTA buffer (kit component) 1:10 (1 volume + 9 volumes) with distilled or deionized water just prior to use - final pH should be 6.5 (±0.1). It is important to check quality of deionized water before dilution, since if pH is not 6.5 (±0.1) solution should be discarded. A second aliquot should then be prepared with deionized water. Keep tightly closed. No need to adjust pH of reconstituted versene buffer.
2. Label 5 pairs of 1.5 x 15 cm or 1.2 x 12 cm test tubes with CEA standard dose level to be used in each tube. (It is suggested that 0, 10, 25, 50 and 100 µl of the standard, which is equivalent to 0, 1.25, 3.125, 6.25 and 12.5 ng of CEA activity, be used).
3. Add 5 ml of diluted stock EDTA buffer to each tube.
4. Add proper amount of CEA standard (10, 25, 50 and 100 µl) to each tube, and mix contents of tubes on Vortex-type mixer. From this point the tubes are treated the same as the tubes containing the plasma extracts.

Assay of Extracts for CEA Content:

Treat both sets of tubes - those containing the plasma extracts and those set up for preparation of the standard curve - in exactly the same manner.

In the following steps (addition of antiserum, isotope-labeled CEA, and Z-gel) it is essential that the following sequence be maintained: (1) the time required for the addition of any reagent must be the same for all [no more than 60 seconds]; (2) the sequence of adding reagent to the tubes in the rack must be consistent. The most critical step is the incubation time following the addition of the isotope-labeled CEA. Tubes should be batched as one person cannot handle a large number of tubes at one time since the time for dispensing reagent and mixing is limited to one minute. The addition of Z-gel will stop most of the reaction if dispensed in a manner to intermix with the contents in the tubes. This allows adding Z-gel to all the tubes before vortex mixing. Low plasma CEA values will result if the material for the inhibition assay is incubated for a shorter period than the extracts of plasma. High plasma CEA values will result if the material for the inhibition assay is incubated for a longer period than the plasma extracts.

Add 25 µl of CEA antiserum to each tube. Either an automatic diluter or pipette may be used. Mix contents on a Vortex type mixer. Place tubes in a 45°C (±1°) circulating water bath for 30 (±1) minutes. If water bath is covered, stopper tubes to prevent water from condensing inside tubes.

Remove tubes from 45°C water bath and add 25 µl of $^{125}$I-CEA to each. Mix contents with a Vortex-type mixer. Return tubes to water bath for another 30 (±1) minutes. Remove tubes and immediately add 2.5 ml of zirconyl phosphate gel (Z-gel) to each tube. It is important that the Z-gel be shaken vigorously before use to assure homogeneity and added rapidly with an automatic dispensing instrument so that the reaction is kept constant in all tubes. At this point the $^{125}$I-CEA bound to the antibody is separated from the excess free $^{125}$I-CEA. Vortex and centrifuge immediately at 1000 x g for 5 minutes (tubes smaller than 1.2 x 12 cm must be covered before mixing).

Gently decant supernatants into an isotope waste container, and blot tube tops with paper towels before returning tubes to upright position. Add 5 ml of pH 6.25 ammonium acetate (0.1 M acetate) solution, to each tube. Completely disperse gel by using a Vortex-type mixer. Careful attention to mixing at this time is necessary to assure proper washing. Centrifuge again at 1000 x g for 5 minutes. Again gently decant supernatants into an isotope waste container and blot tube tops with paper towels.

When gently decanting the supernatant fluid from the Z-gel pellet, care should be taken so that once the tube is inverted during decanting it remains in the inverted position for blotting. This single inversion preserves the Z-gel pellet configuration. Do not shake the tubes since this will force the Z-gel up the sides of the tube and cause scintillation counting errors.

Wash outside of tubes by dipping rack of tubes into a clean water bath. Wipe tubes individually prior to positioning in the counter. This step prevents erroneous counts and counter contamination by any isotope contamination on the outside tube wall. The Z-gel pellet is now ready to be counted.

Counting:

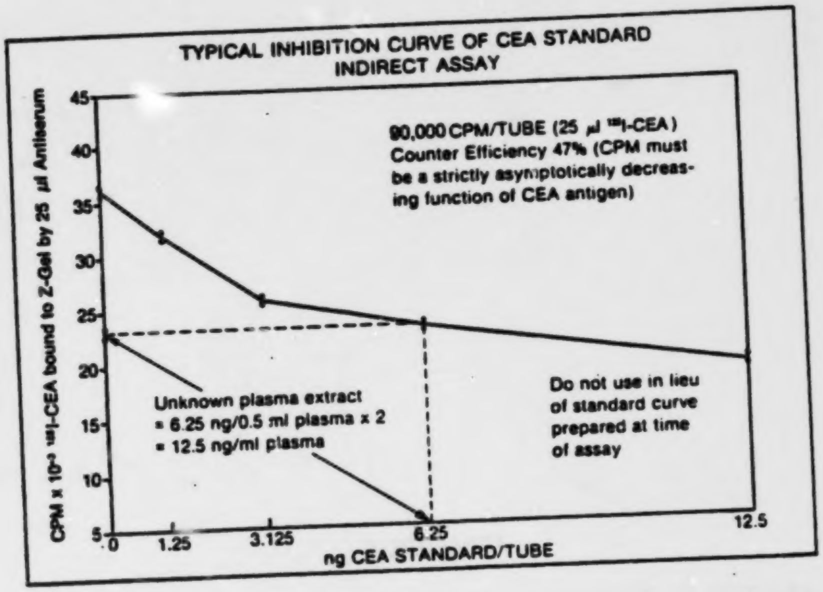
Determine the amount of bound $^{125}$I-CEA by placing tube in a gamma scintillation spectrometer and counting for one minute. This information may be recorded by computer. A typical print-out shows the specimen number in the first column, the time it was counted in the second column, and the counts per minute in the third column. This information is used to construct a standard curve - plot the mean CPM of $^{125}$I-CEA bound to the Z-gel against the antigen dose in each tube (see Figure J-1). Duplicates should be within 5% of the mean CPM.

## 8.0 Calculations and Interpretation

The CEA content of each extract is determined from a graph prepared by plotting CPMs *vs*. ng of CEA in standards. Values must be multiplied by two to convert to ng of CEA/ml of plasma. CPM values greater than the 0 antigen dose are reported as 0 ng of CEA/ml of plasma. Values above 20 ng/ml obtained by this indirect assay are not quantitatively accurate.

## References

J1. "Procedure Manual for CEA-ROCHE Carcinoembryonic Antigen Assay" Roche Diagnostics, Division of Hoffmann-La Roche Inc., Nutley, NJ 07110.



**Sample calculation:**

0.5-ml sample result 23,500 CPM = 6.25 ng CEA
6.25 ng CEA/0.5 ml x 2 = 12.5 ng CEA/ml

Figure J-1. Typical inhibition curve of CEA standard indirect assay.

# APPENDIX K

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS PROCEDURE FOR BREATH SAMPLES

# ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS PROCEDURE FOR BREATH SAMPLES

## 1.0 Principle of the Method

The breath sample is collected on Tenax GC using a specially designed spirometer and low hydrocarbon air "ultrapure" or equivalent. The Tenax cartridge is then dried over $CaSO_4$ and analyzed by thermal desorption into a gas chromatograph with a SCOT capillary column and mass spectrometry (GC/MS).

## 2.0 Apparatus

Spirometer - as diagrammed in Figure K-1. The valves in the Douglas mouth piece must be replaced with Tedlar or similar material. A bubbler filled with distilled deionized water is placed inline with the air tank to humidify the air for subject comfort.

The gas chromatograph/mass spectrometer/computer and thermal desorption are described in Appendix A.

## 3.0 Materials

Tenax GC cartridges
Culture tubes
$CaSO_4$ anhydrous and hydrocarbon free (treated in a muffle furnace at 400°C for 2 hrs)
Ultrapure air

## 4.0 Procedure

The subject is seated in a comfortable chair and the mouthpiece height adjusted to a convenient level. A long spring clamp is used to seal the air flow from Bag A to the mouthpiece. A plug is placed in the mouthpiece opening until the test begins to prevent room air contamination. Air flow from the air tank is started and when the 50 l Bag A is about half full, the mouthpiece is attached, the clamp and plug removed and the subject may begin to breathe on the apparatus. The nose clips must also be in place at this time. After a minute or two, the Nutech sampler pump is started with the flow at approximately 7 l/min. The flow may be adjusted to match the individual subject's respiration rate. It is useful to furl Bag B using spring clips to avoid using this bag as an exponential dilutor but retaining the safety factor of the 50 l bag capacity. After a predetermined volume (75 l)

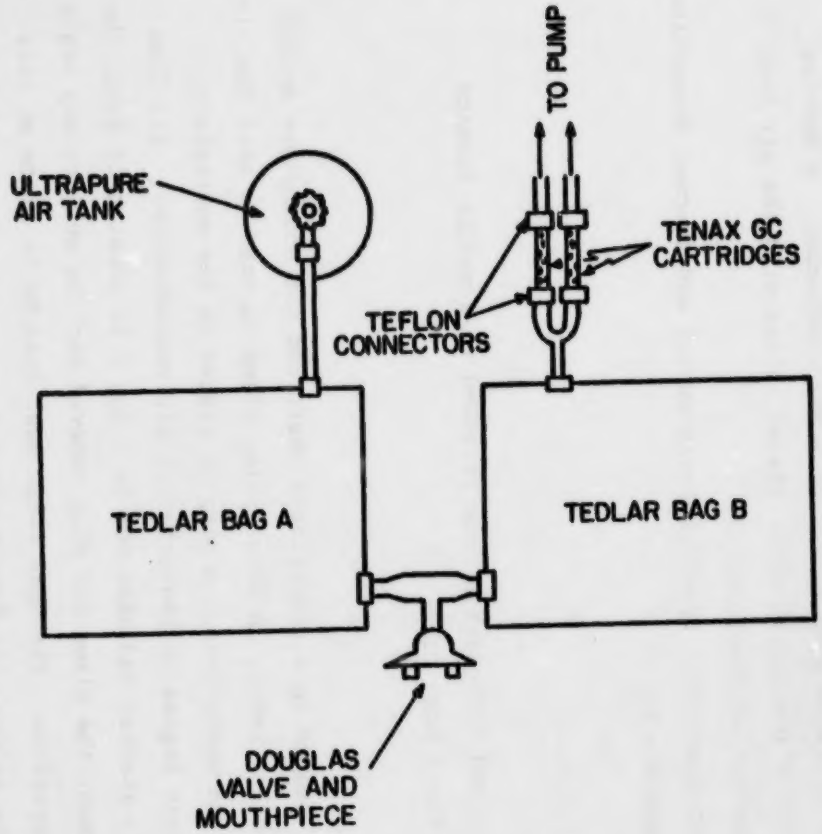

TO PUMP
ULTRAPURE AIR TANK
TENAX GC CARTRIDGES
TEFLON CONNECTORS
TEDLAR BAG A
TEDLAR BAG B
DOUGLAS VALVE AND MOUTHPIECE

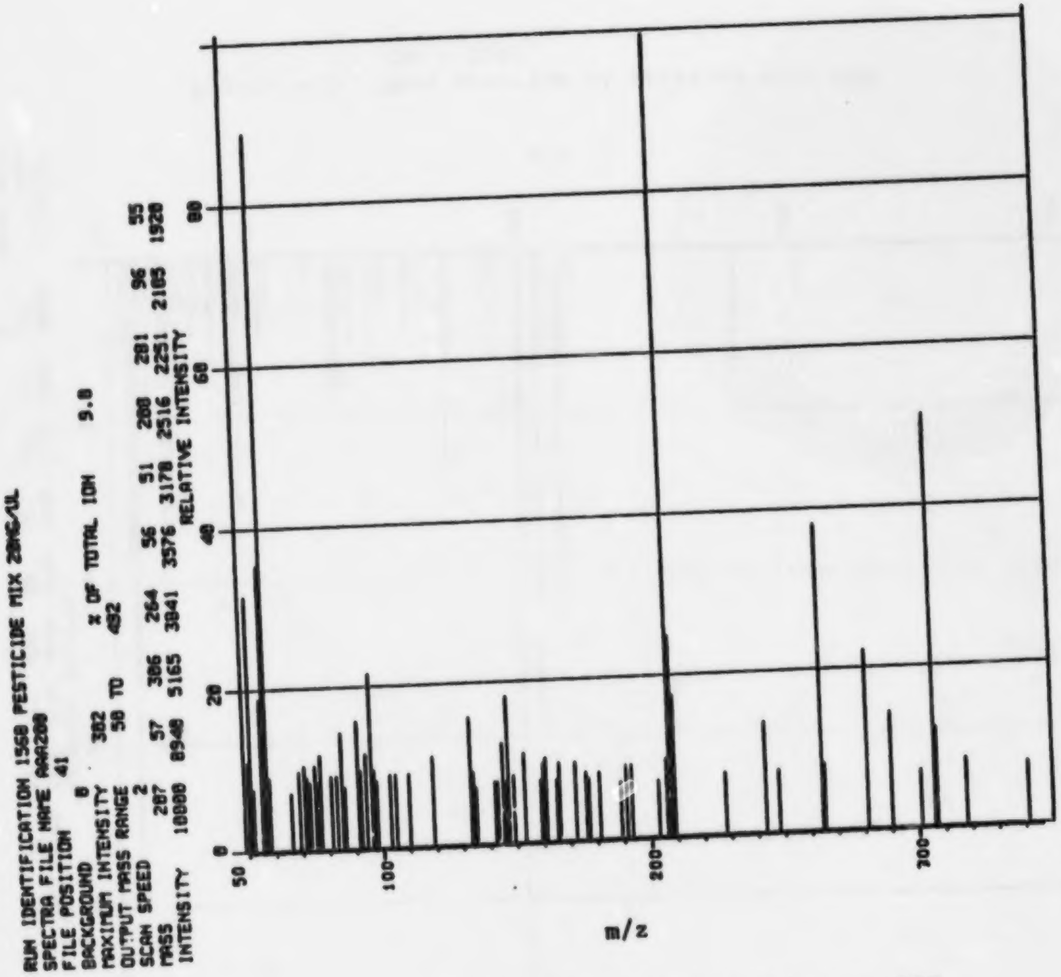
Figure K-1. Schematic of Spirometer for collection of breath samples.

of breath has been sampled, the test is terminated. The subject is removed from the apparatus and the nose clips removed from the subject. The Tenax cartridges are removed and stored in culture tubes. The entire apparatus is then flushed with pure air to decontaminate it for the next use. This is best done by successively filling and evacuating the bags. The mouthpiece is sterilized by placing in alcohol after each use. The Tenax cartridges are desiccated over $CaSO_4$ before analysis by placing $\sim$2 g in the bottom of a culture tube and covering with glass wool. The cartridge is then sealed in the culture tube for a minimum of 1 hr.

Analysis of the Tenax cartridge performed as for air samples (Appendix A).

# APPENDIX L

# GC/MS/COMP LIMIT OF DETECTION DATA: MASS SPECTRA, ION TRACINGS, AND SIM PLOTS



```
RUN IDENTIFICATION 1550 PESTICIDE MIX 20NG/UL
SPECTRA FILE NAME AAA200
FILE POSITION      41
BACKGROUND         0
MAXIMUM INTENSITY      302          x OF TOTAL ION    9.0
OUTPUT MASS RANGE       50  TO  492
SCAN SPEED         2
MASS              207   57   306  264  56   51   200  281  96   55
INTENSITY       10000  8948 5165 3841 3576 3170 2516 2251 2105 1920
```

Figure L-1. Mass spectrum of trifluralin near LOD (MW = 335).

```
RUN IDENTIFICATION 1560 PESTICIDE MIX 20NG/UL
SPECTRA FILE NAME AAA199
FILE POSITION      52
BACKGROUND          0
MAXIMUM INTENSITY    400          % OF TOTAL ION     0.2
OUTPUT MASS RANGE     50 TO    492
SCAN SPEED          2
MASS             200    57   207    58    68   202   215    55    56    69
INTENSITY      10000  7043  5931  5294  4019  4019  3970  3823  3400  3106
```

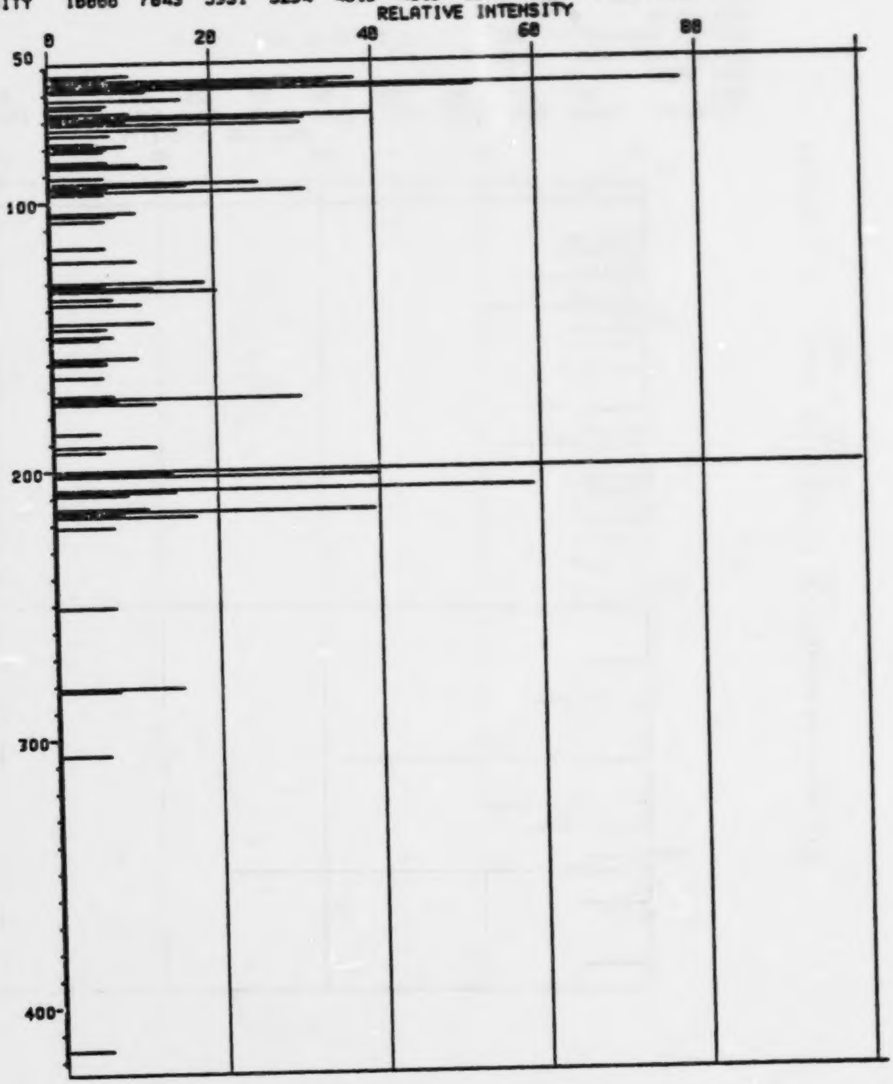


Figure L-2. Mass spectrum of atrazine near LOD
(MW = 216).

```
RUN IDENTIFICATION 1568 PESTICIDE MIX 20NG/UL
SPECTRA FILE NAME AAA199
FILE POSITION     62
BACKGROUND         0
MAXIMUM INTENSITY    274         % OF TOTAL ION      4.7
OUTPUT MASS RANGE     50 TO .  492
SCAN SPEED         2
MASS         183   219   207   181   111    51   217   189    96    50
INTENSITY  10000  9635  8321  7737  7445  7299  6423  5693  5328  5255
```

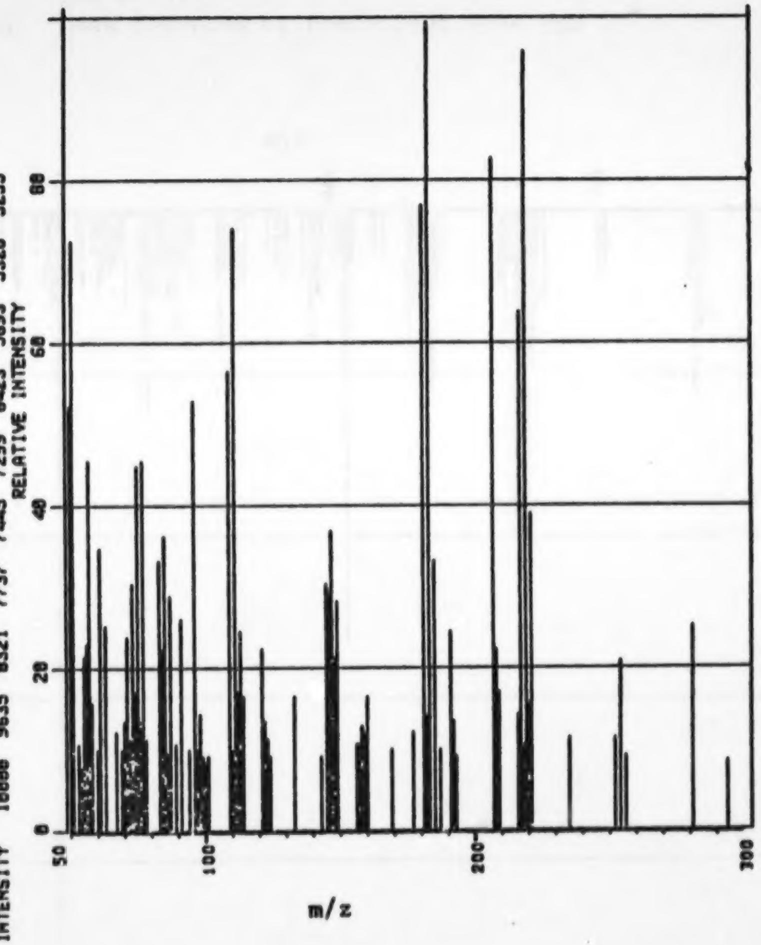
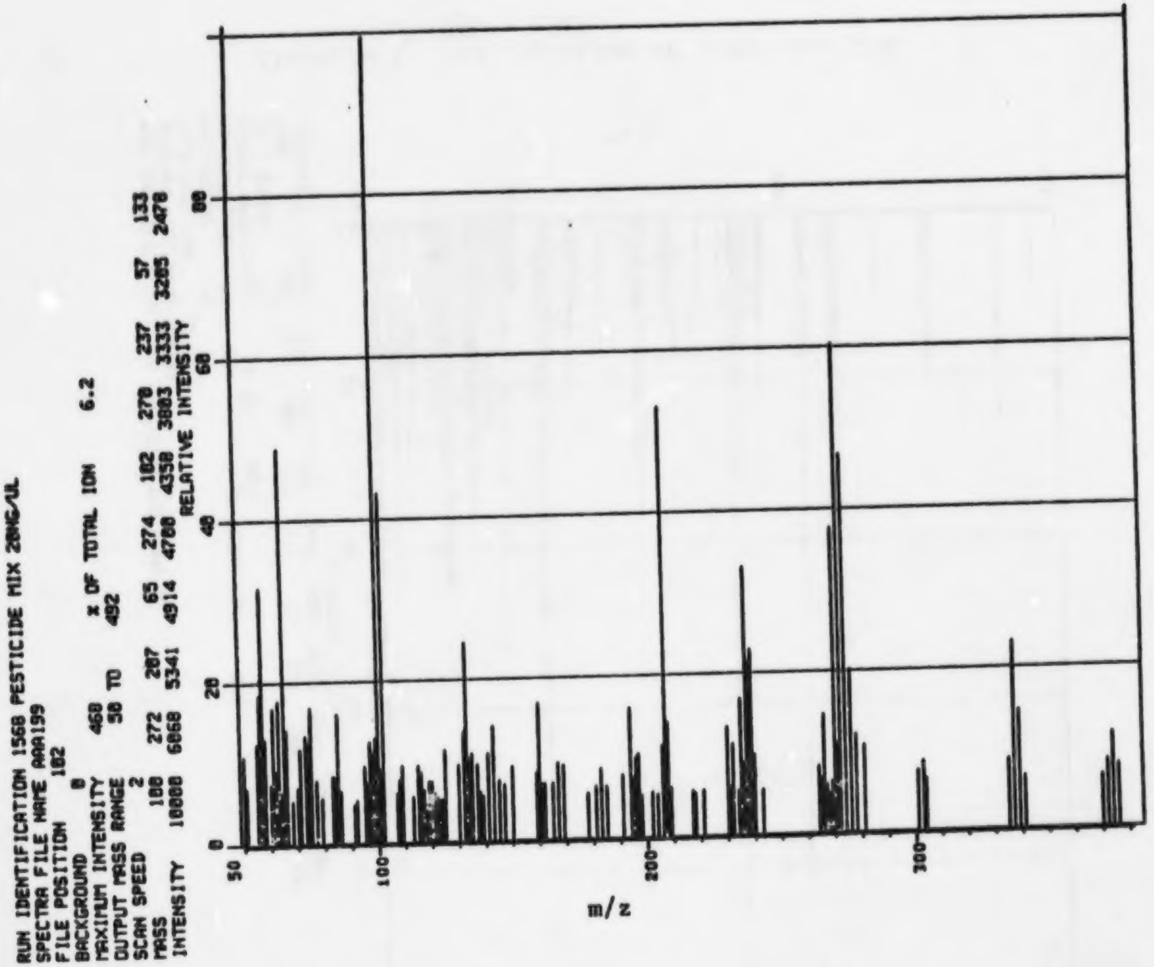
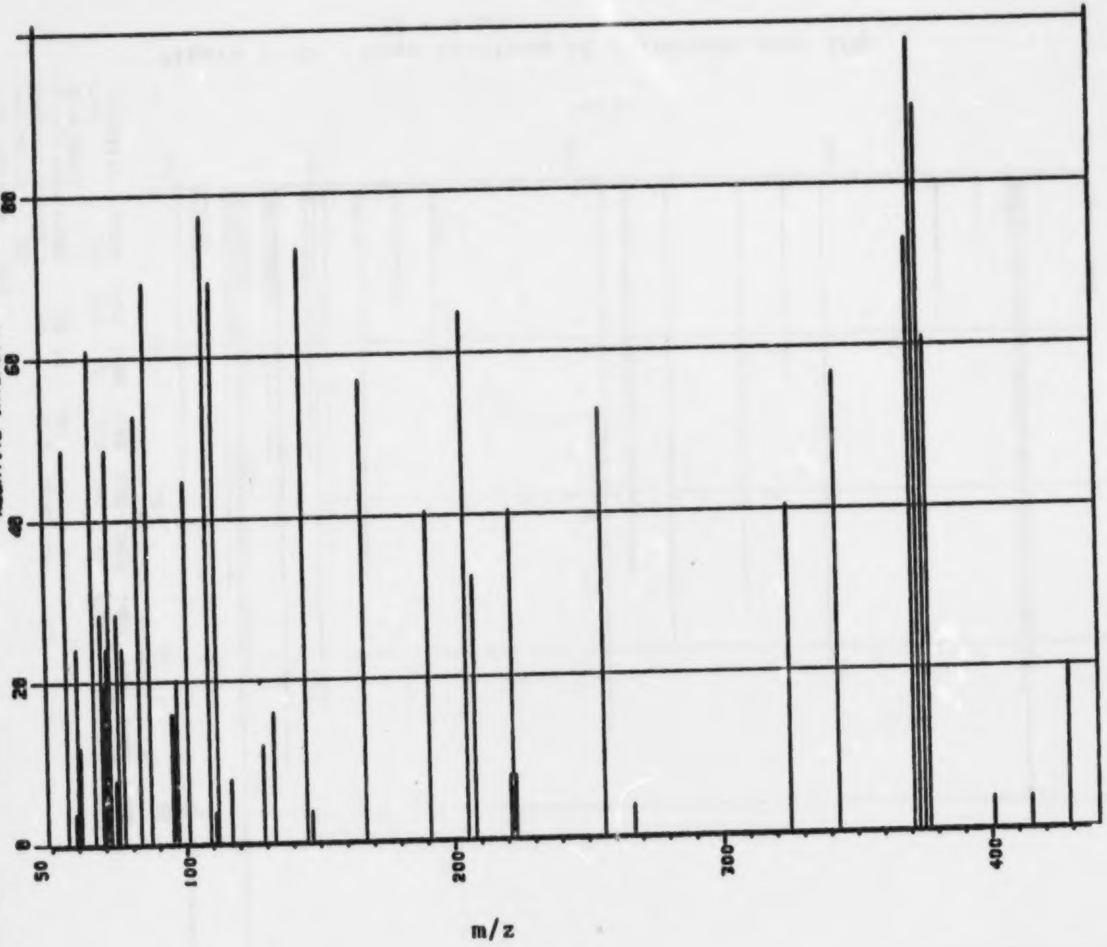


Figure L-3. Mass spectrum of γ-BHC near LOD (MW = 291).



```
RUN IDENTIFICATION 1568 PESTICIDE MIX 20NG/UL
SPECTRA FILE NAME AAA199
FILE POSITION    182
BACKGROUND         0
MAXIMUM INTENSITY     468         % OF TOTAL ION      6.2
OUTPUT MASS RANGE     50  TO  492
SCAN SPEED         2
MASS         100    272   207   65    274   182   270   237   57    133
INTENSITY  10000   6868  5341  4914  4780  4358  3803  3333  3285  2478
```

Figure L-4. Mass spectrum of heptachlor near LOD (MW = 373).



```
RUN IDENTIFICATION 1568 PESTICIDE MIX 100NG/UL
SPECTRA FILE NAME AAA194
FILE POSITION     214
BACKGROUND      185
MAXIMUM INTENSITY      48          % OF TOTAL ION     6.2
OUTPUT MASS RANGE      50  TO    452
SCAN SPEED        2
MASS         373   375   189   145   371    87   112   205    66   377
INTENSITY  10000  9166  7916  7500  7500  7083  7083  6666  6250  6250
```

Figure L-5a.  Mass spectrum of chlordane near LOD.
(MW = 410).

```
RUN IDENTIFICATION 1560 PESTICIDE MIX 100NG/UL
SPECTRA FILE NAME AAA194
FILE POSITION    233
BACKGROUND     185
MAXIMUM INTENSITY      50          % OF TOTAL ION      6.2
OUTPUT MASS RANGE      50  TO    492
SCAN SPEED        2
MASS             70   373    87   212   181   371    72   297   375   237
INTENSITY     10000  8400  7600  7200  6800  6800  6400  6400  6400  6000
```

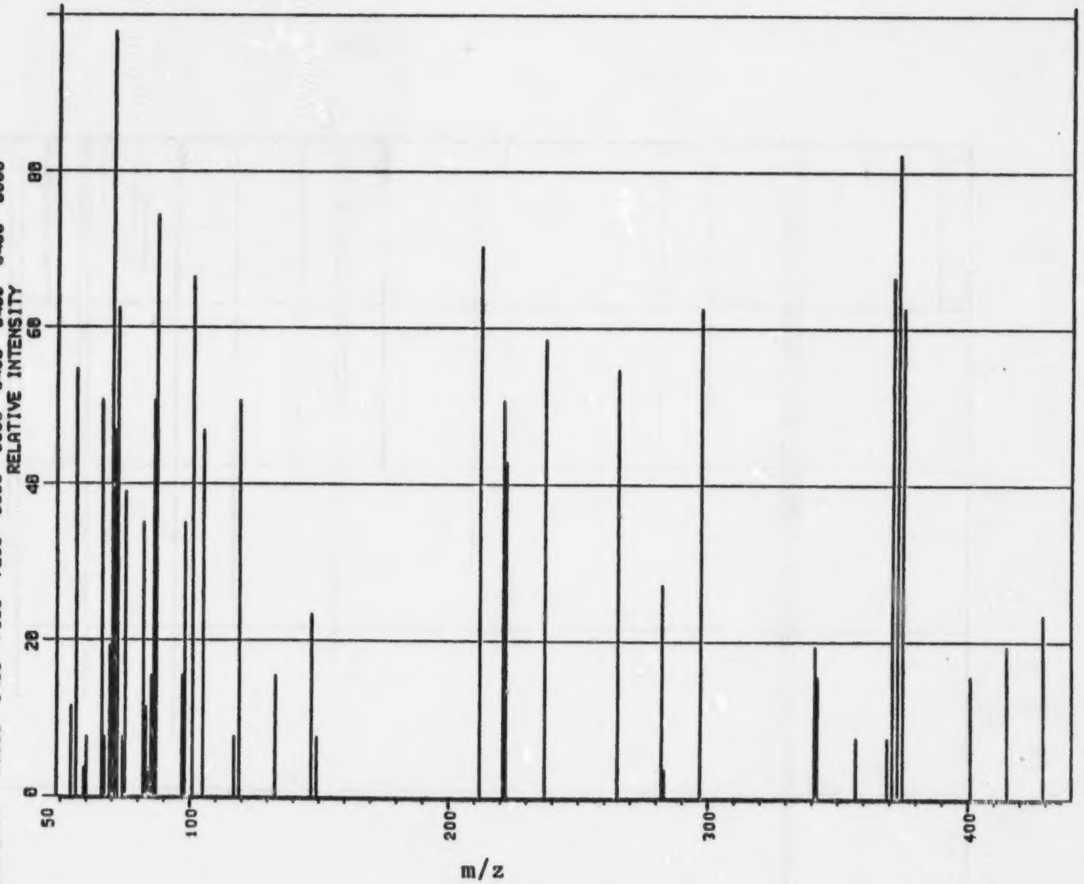
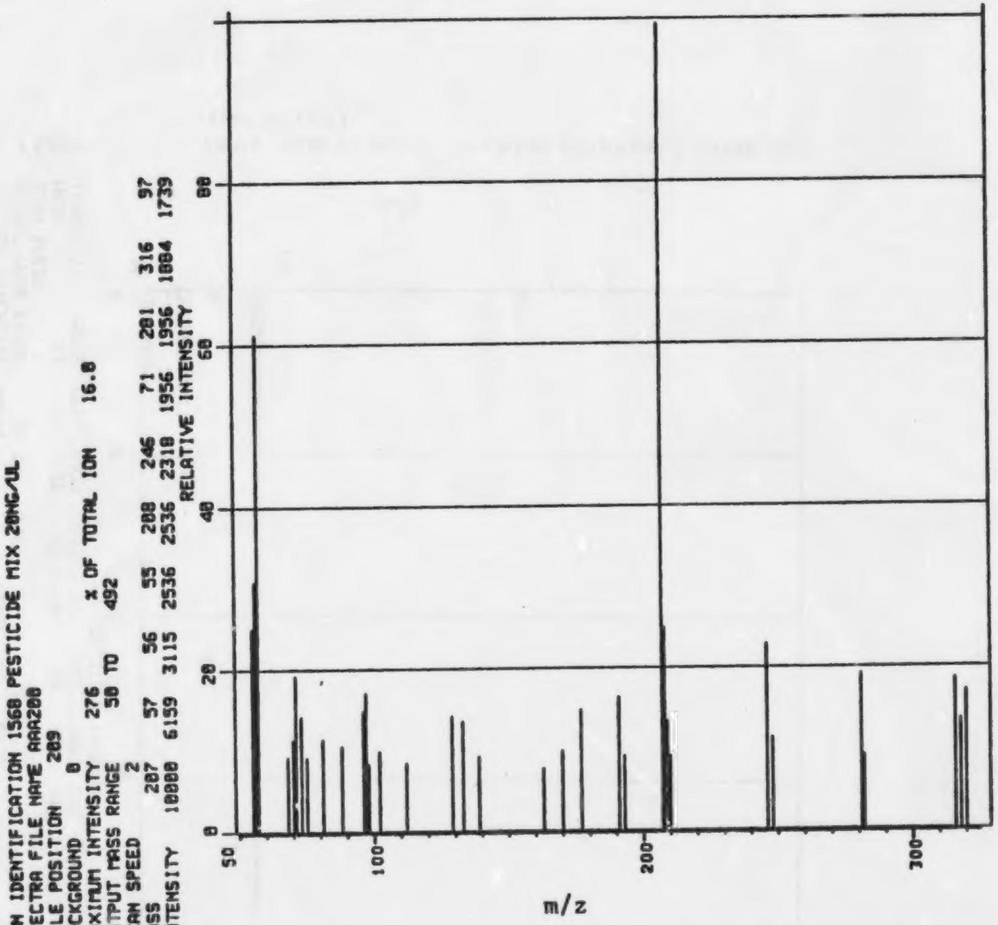


Figure L-5b. Mass spectrum o. chlordane near LOD (MW = 410).



```
RUN IDENTIFICATION 1568 PESTICIDE MIX 20NG/UL
SPECTRA FILE NAME AAA200
FILE POSITION    209
BACKGROUND         0
MAXIMUM INTENSITY  276      % OF TOTAL ION    16.0
OUTPUT MASS RANGE   50 TO   492
SCAN SPEED         2
MASS          207    57    56    55   208   246    71   281   316    97
INTENSITY   10000  6159  3115  2536  2536  2318  1956  1956  1884  1739
```

Figure L-6. Mass spectrum of p,p'-DDE near LOD (MW = 318).

```
RUN IDENTIFICATION 2.08NG 2ECLBIPHENYL: 11.4NG CL6BIPHENYL: 23.1NG CL10BIPHENYL
SPECTRA FILE NAME AAA176
FILE POSITION     13
NO BACKGROUND SUBTRACTION
MAXIMUM INTENSITY *****           x OF TOTAL ION    35.0
OUTPUT MASS RANGE      50 TO     740
SCAN SPEED         2
MASS              91     92     65     63     51     50     93    188     89     62
INTENSITY      10000   7592   1430   1187    997    641    590    532    507    473
```

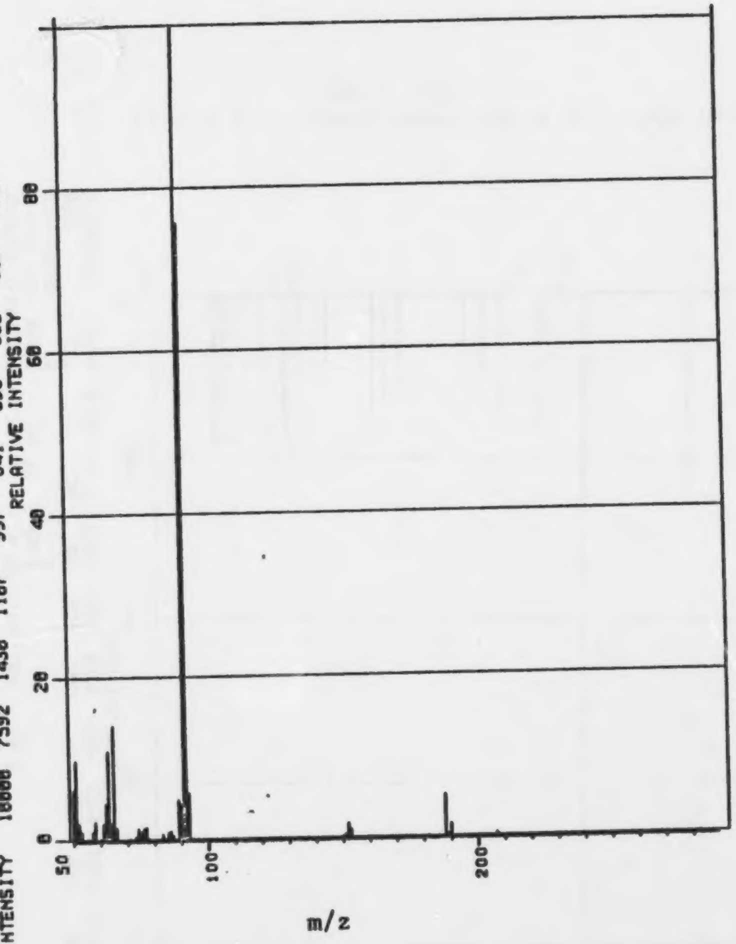
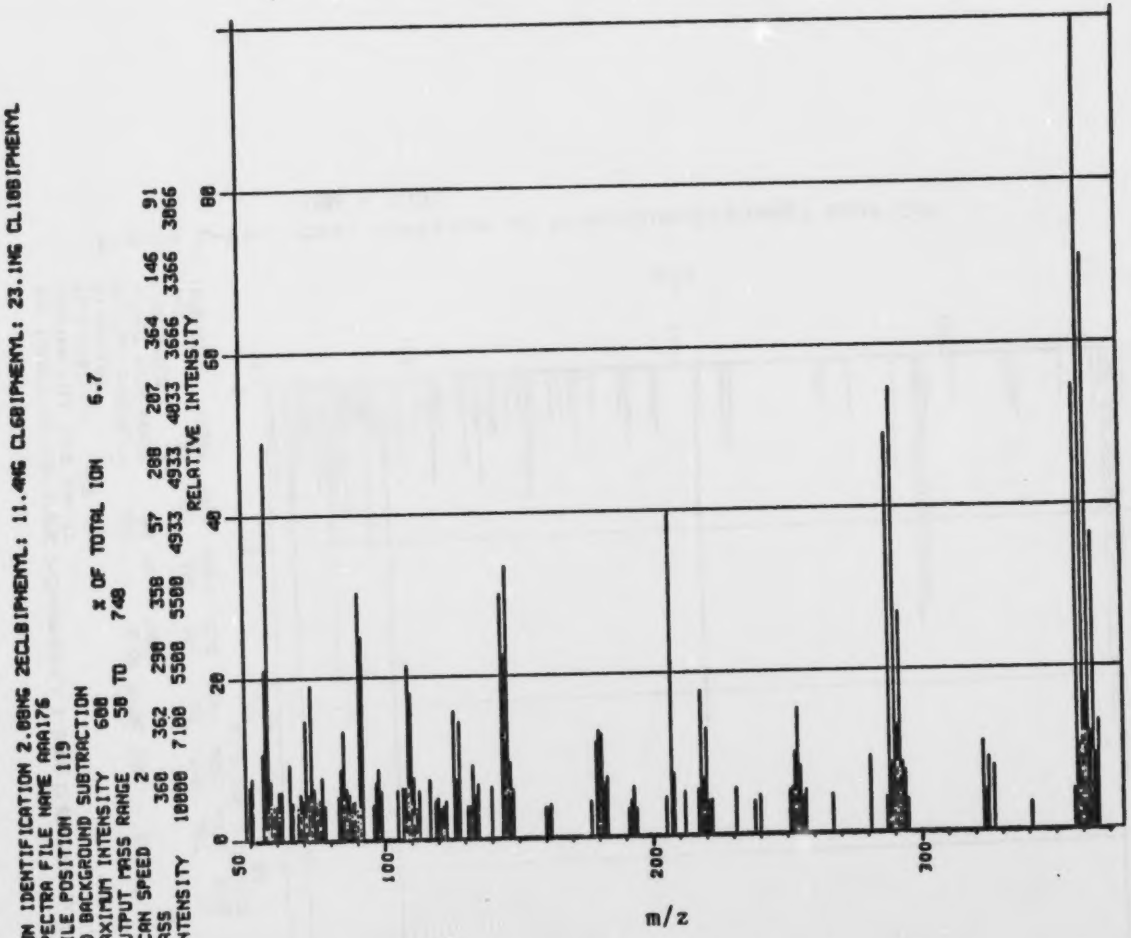


Figure L-7. Mass spectrum of 2-chlorobiphenyl near LOD (MW = 188).



```
RUN IDENTIFICATION 2.08NG 2ECLBIPHENYL: 11.4NG CL6BIPHENYL: 23.1NG CL10BIPHENYL
SPECTRA FILE NAME AAA176
FILE POSITION     119
NO BACKGROUND SUBTRACTION
MAXIMUM INTENSITY     600           % OF TOTAL ION     6.7
OUTPUT MASS RANGE      50  TO  740
SCAN SPEED              2
MASS          360   362   298   358    57   288   207   364   146    91
INTENSITY   10000  7100  5500  5500  4933  4933  4033  3666  3366  3066
```

Figure L-8a. Mass spectrum of hexachlorobiphenyl near LOD (MW = 358).

```
RUN IDENTIFICATION 370PG 2-CLBIPHENYL: 1.04NG CL6BIPHENYL: 2.10NG CL10BIPHENYL:0
SPECTRA FILE NAME AAA177
FILE POSITION    110
BACKGROUND       0
MAXIMUM INTENSITY    456        % OF TOTAL ION     6.5
OUTPUT MASS RANGE     50 TO    740
SCAN SPEED       2
MASS            57    91   360   362    92   358   207    71   288   290
INTENSITY    10000  9870  8201  5219  5043  4685  4429  3720  3289  3157
```

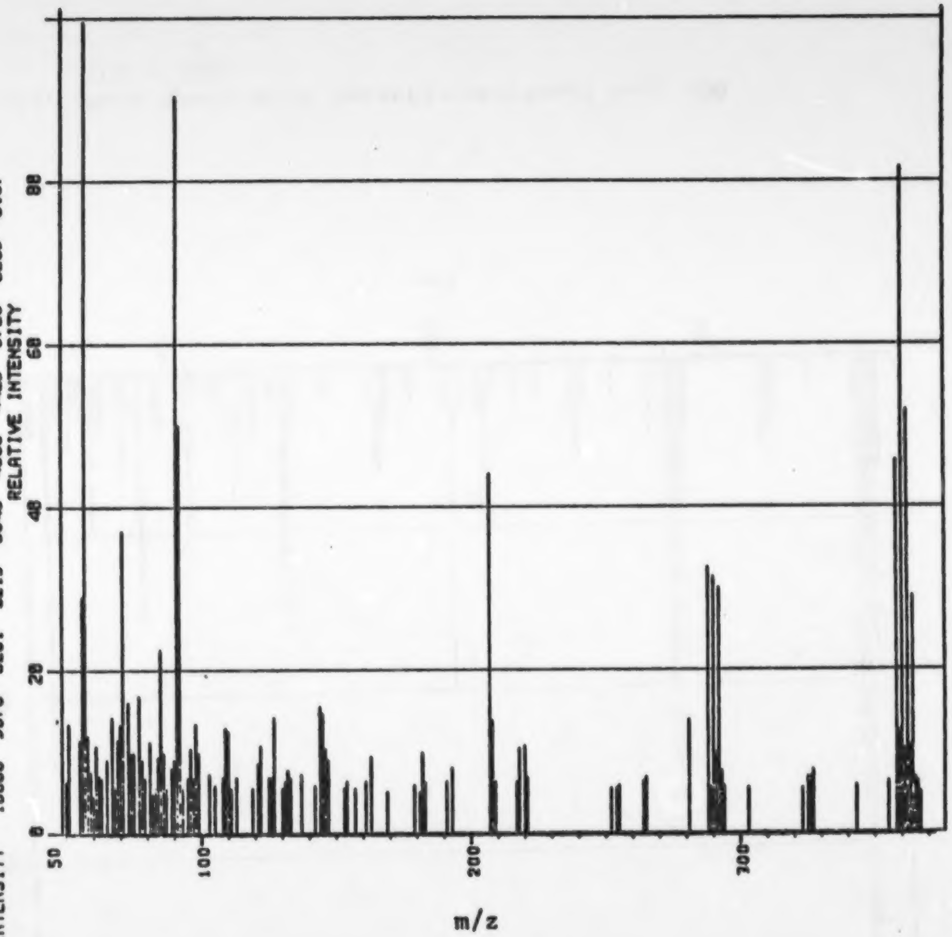


Figure L-8b. Mass spectrum of hexachlorobiphenyl near LOD (MW = 358).

```
RUN IDENTIFICATION 2.08NG 2ECLBIPHENYL: 11.4NG CL6BIPHENYL: 23.1NG CL10BIPHENYL
SPECTRA FILE NAME AAAI76
FILE POSITION     535
NO BACKGROUND SUBTRACTION
MAXIMUM INTENSITY    498            % OF TOTAL ION     5.7
OUTPUT MASS RANGE     50  TO    748
SCAN SPEED             2
MASS                  57    498    500    287    496    501     71    428     69    430
INTENSITY          10000   7586   5278   5123   4581   3842   3793   3596   3399   3399
```

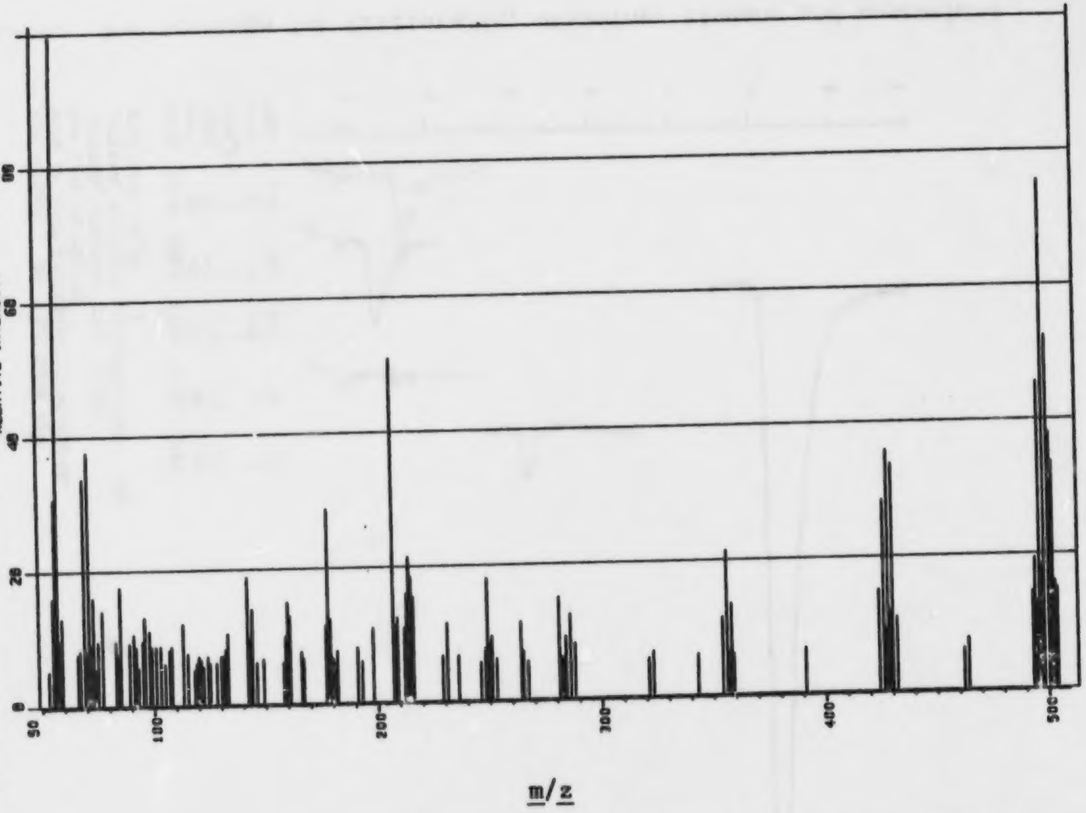


Figure L-9. Mass spectrum of decachlorobiphenyl near LOD (MW = 494).

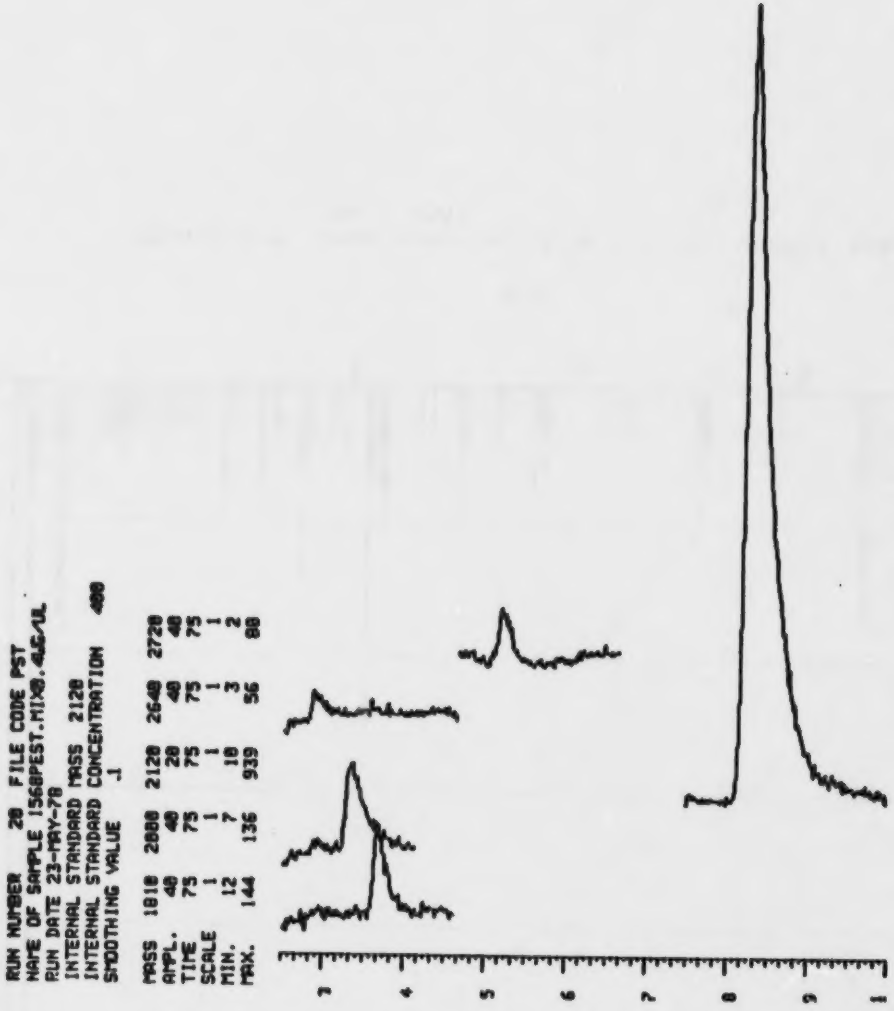
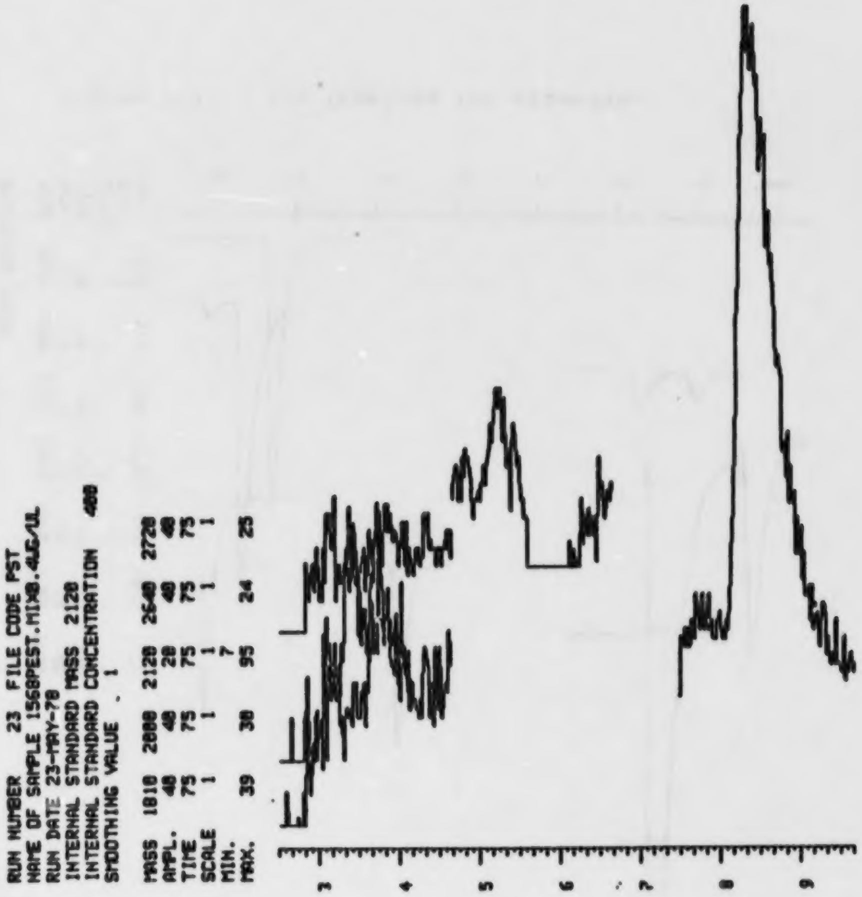


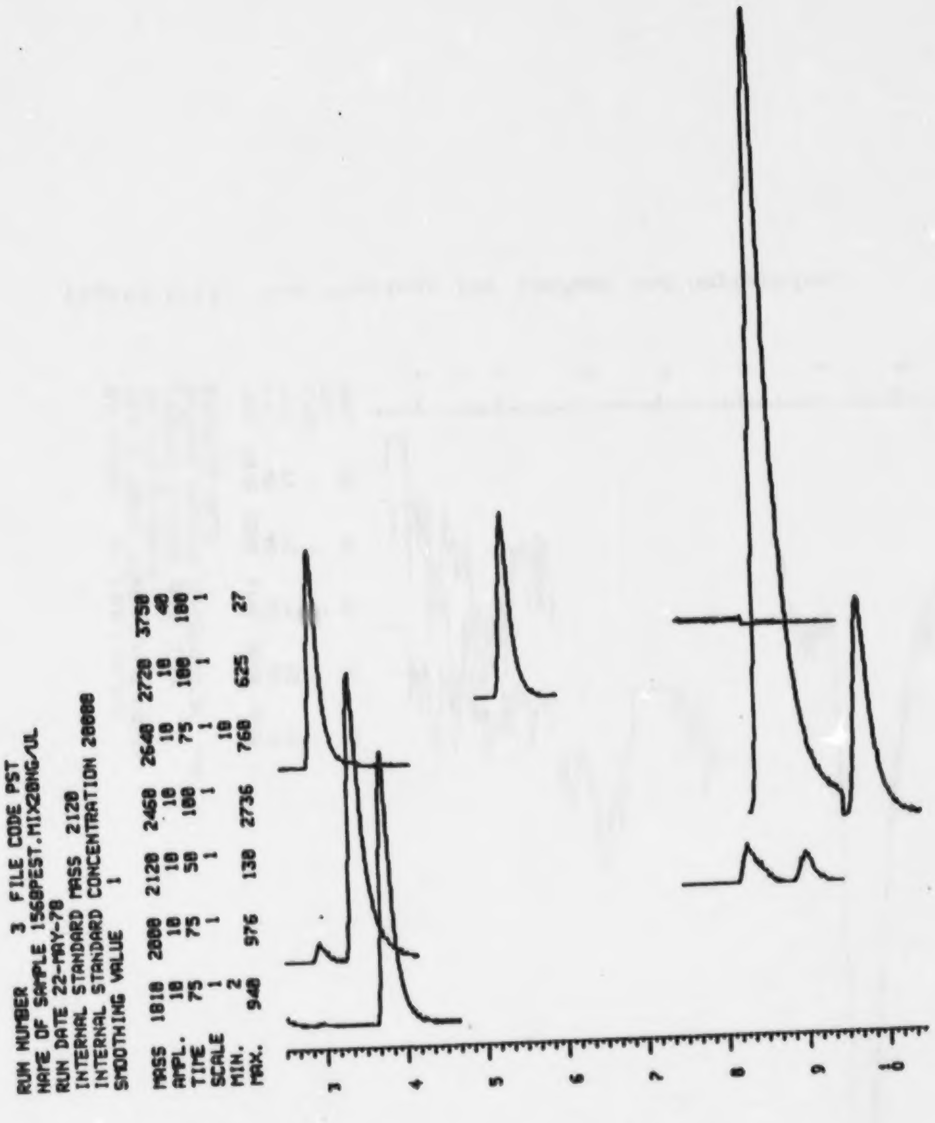
Figure L-10. Ion tracings for trifluralin, atrazine, lindane and heptachlor.



```
RUN NUMBER    23   FILE CODE PST
NAME OF SAMPLE 1568PEST.MIX0.4UG/UL
RUN DATE 23-MAY-78
INTERNAL STANDARD MASS  2120
INTERNAL STANDARD CONCENTRATION   400
SMOOTHING VALUE . 1

MASS   1810  2080  2120  2640  2720
AMPL.   40    40    20    40    40
TIME    75    75    75    75    75
SCALE    1     1     1     1     1
MIN.                 7
MAX.    39    38    95    24    25
```

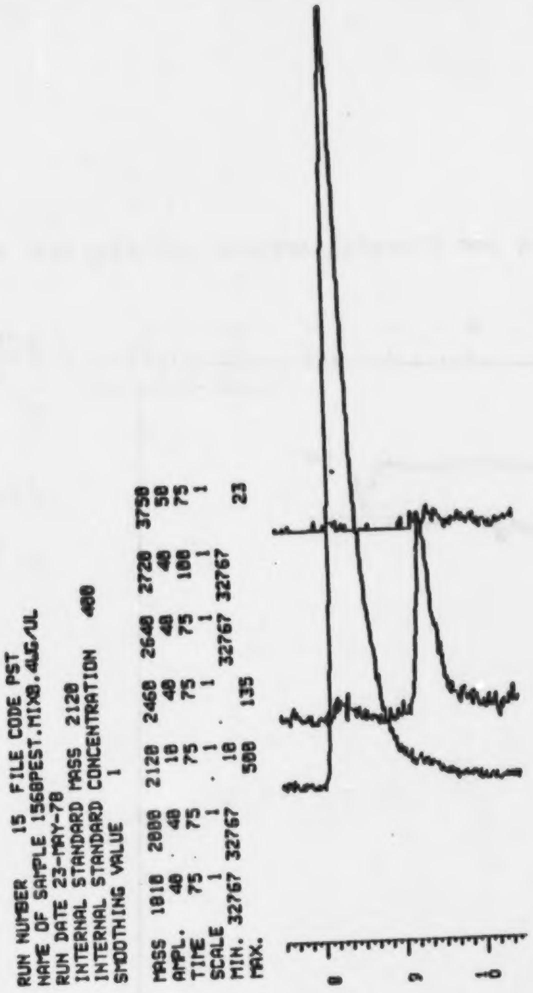
Figure L-11. Ion tracings for lindane and heptachlor.



```
RUN NUMBER       3  FILE CODE PST
NAME OF SAMPLE 1568PEST.MIX20NG/UL
RUN DATE 22-MAY-78
INTERNAL STANDARD MASS  2120
INTERNAL STANDARD CONCENTRATION 20000
SMOOTHING VALUE    1

MASS   1810  2000  2120  2460  2640  2720  3750
AMPL.    10    10    10    10    10    10    40
TIME     75    75    50   100    75   100   100
SCALE     1     1     1     1     1     1     1
MIN.      2                      10
MAX.    940   976   130  2736   760   625    27
```

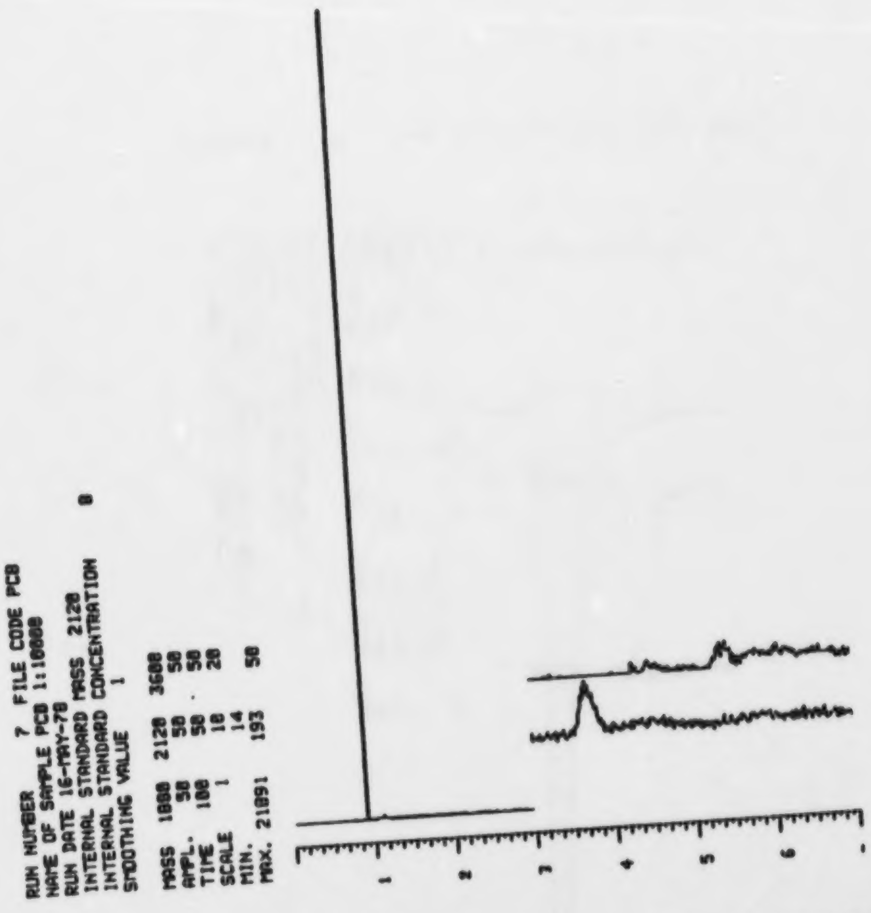
Figure L-12.  Ion tracings for chlordane.



```
RUN NUMBER      15  FILE CODE PST
NAME OF SAMPLE 1560PEST.MIX0.4UG/UL
RUN DATE 23-MAY-78
INTERNAL STANDARD MASS  2120
INTERNAL STANDARD CONCENTRATION    400
SMOOTHING VALUE      1

MASS   1010  2000  2120  2460  2640  2720  3750
AMPL.    40    40    10    40    40    40    50
TIME     75    75    75    75    75   100    75
SCALE     1     1     1     1     1     1     1
MIN.  32767 32767    10       32767 32767
MAX.              500   135                 23
```

Figure L-13. Ion tracings for p,p'-DDE.



```
RUN NUMBER       7  FILE CODE PCB
NAME OF SAMPLE PCB 1:10000
RUN DATE 16-MAY-78
INTERNAL STANDARD MASS  2120
INTERNAL STANDARD CONCENTRATION         0
SMOOTHING VALUE    1

MASS   1880  2120  3600
AMPL.    50    50    50
TIME    100    50 .  50
SCALE     1    10    20
MIN.           14
MAX.  21891   193    50
```

Figure L-14. Ion tracings for 2-chlorobiphenyl and hexachlorobiphenyl.

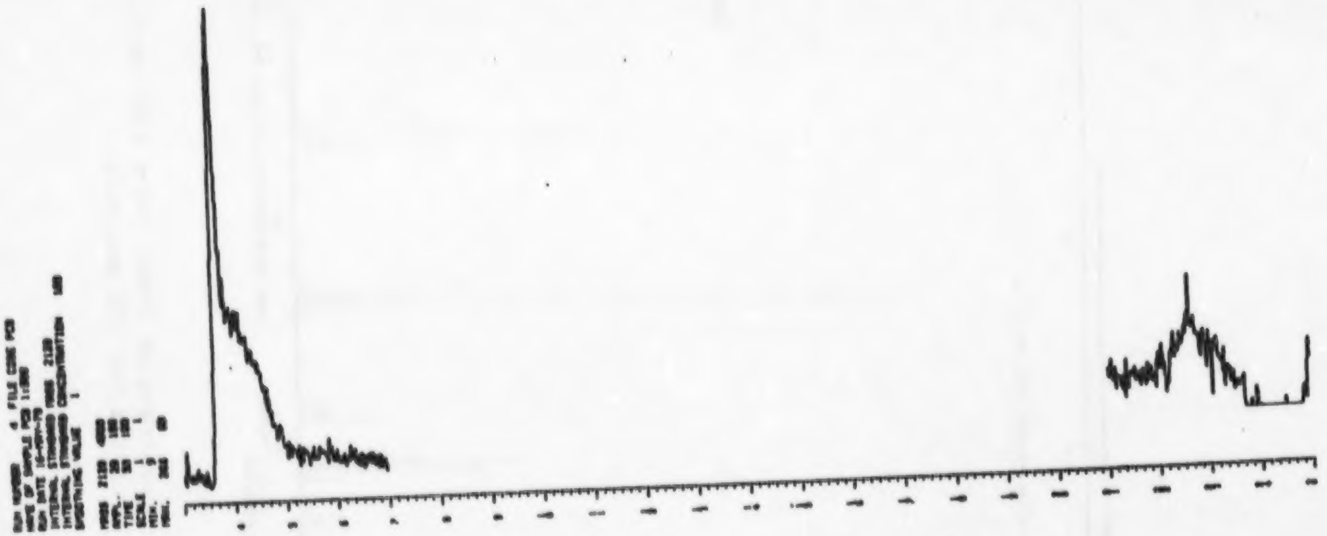


Figure L-15. Ion tracing for decachlorobiphenyl.

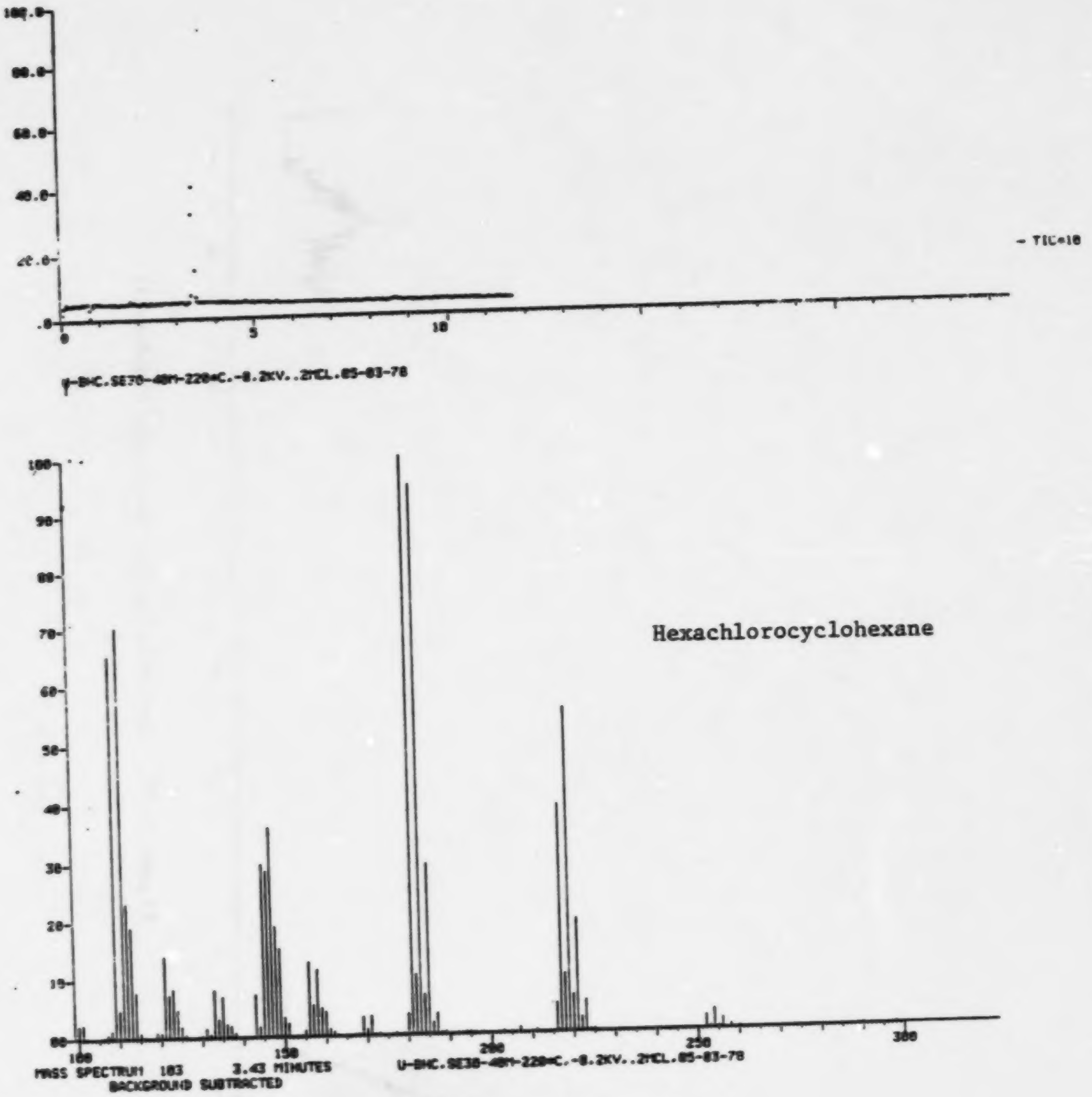


Figure L-16. Analysis of γ-BHC (40 m SCOT capillary, SE-30); m/z 219 chosen for SIM analysis.

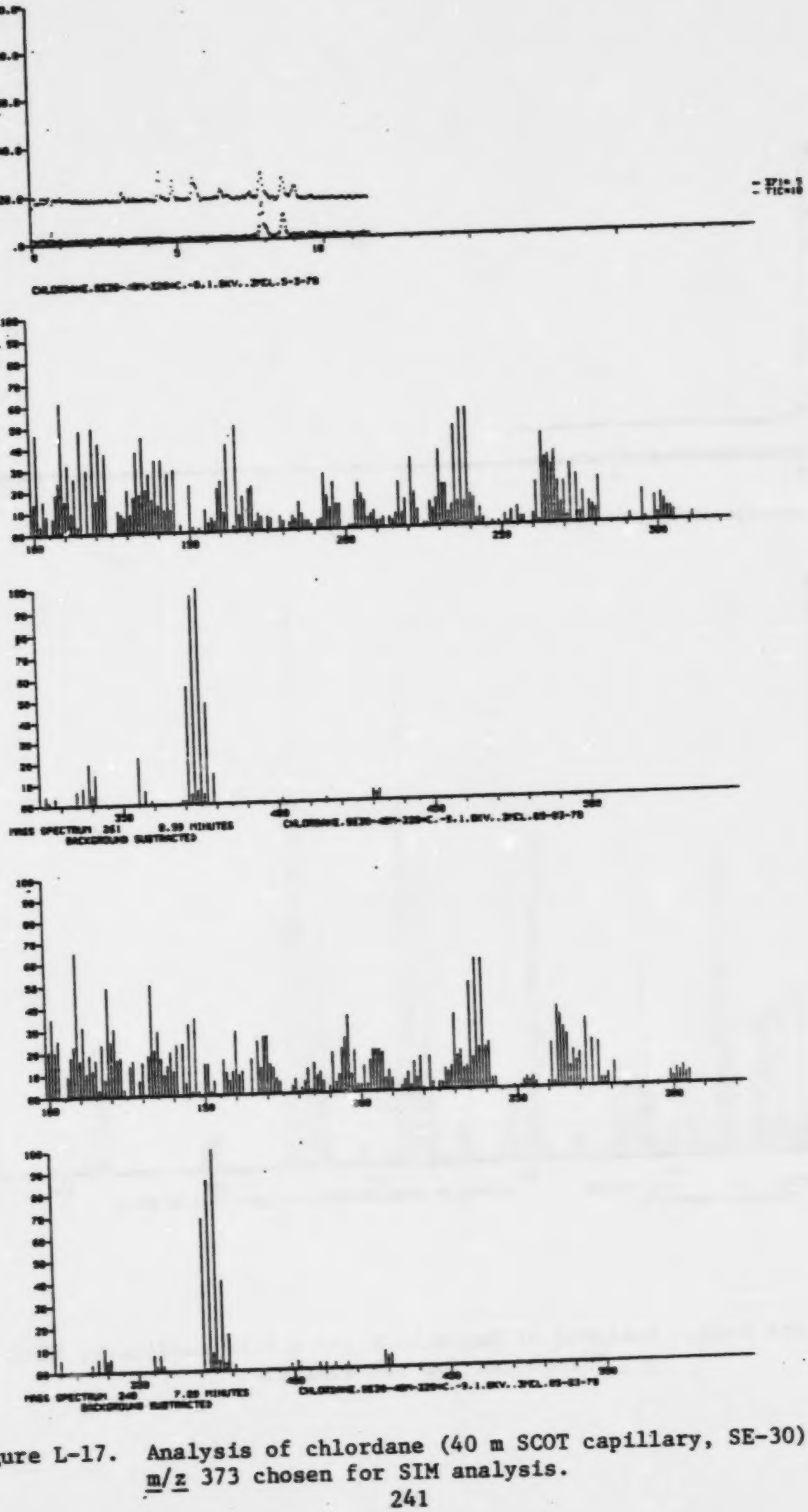


Figure L-17. Analysis of chlordane (40 m SCOT capillary, SE-30); m/z 373 chosen for SIM analysis.

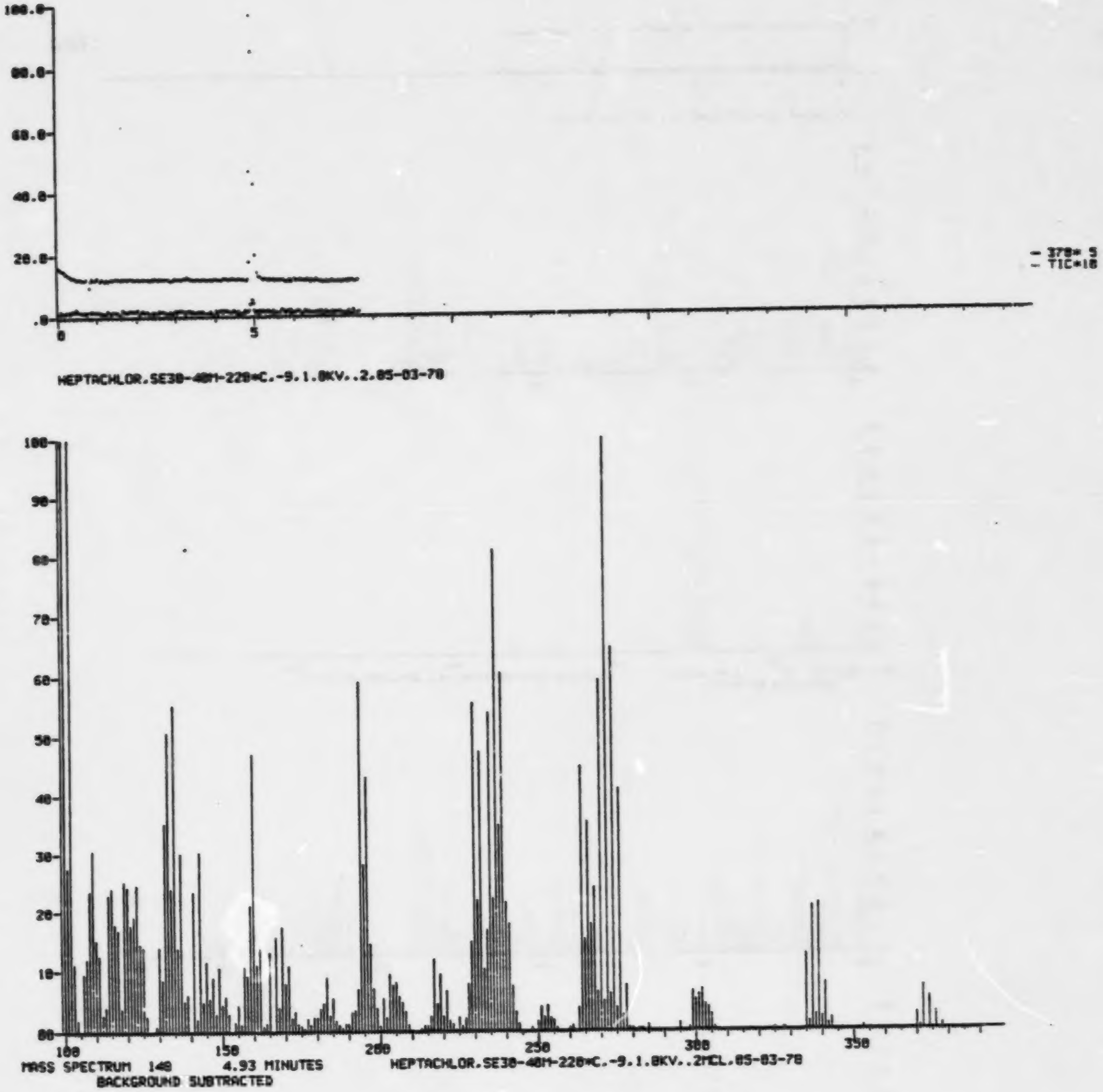


Figure L-18. Analysis of heptachlor (40 m, SCOT capillary, SE-30); m/z 272 chosen for SIM analysis.

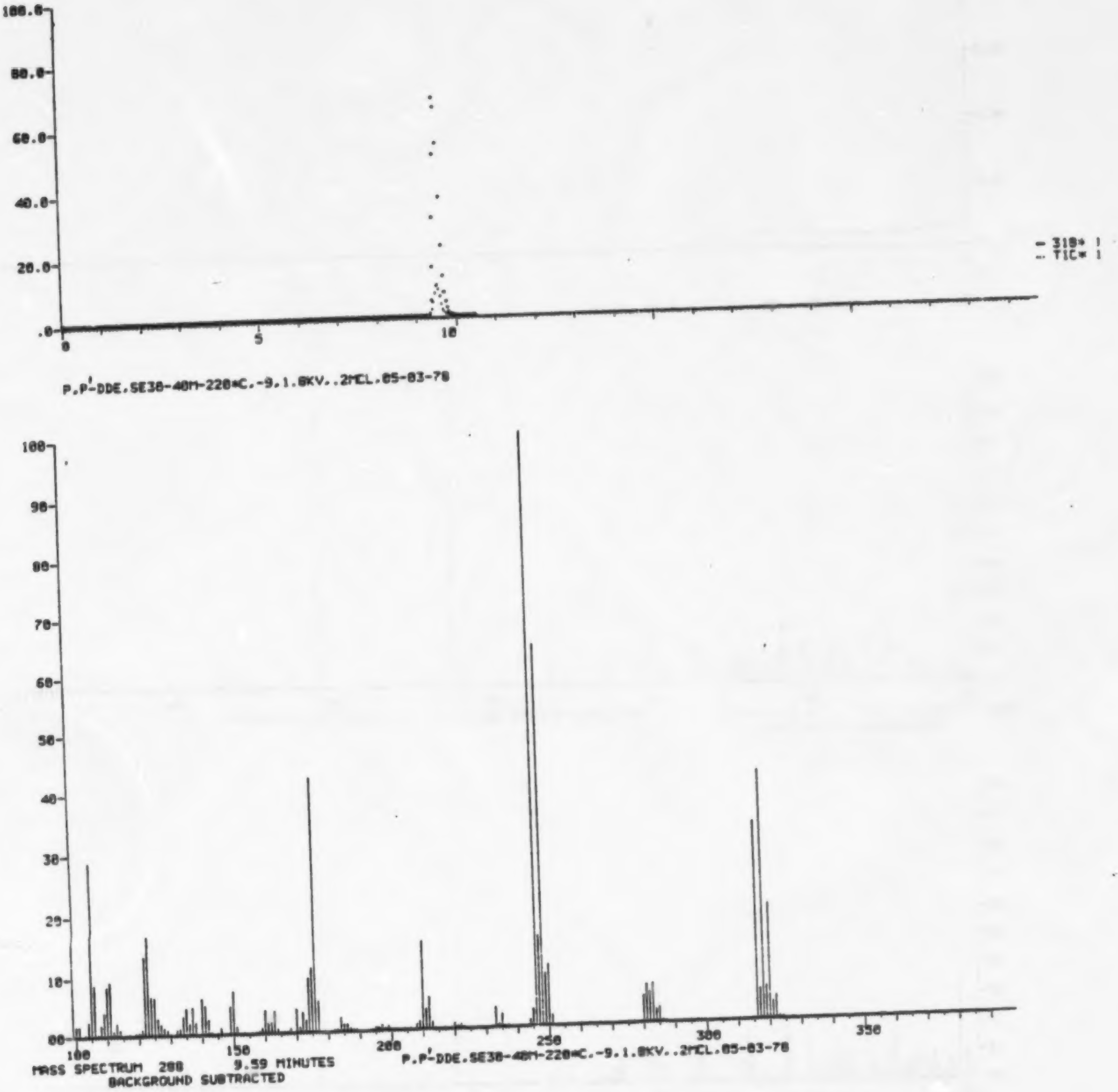


Figure L-19. Analysis of p,p'-DDE (40 m SCOT capillary, SE-30); m/z 246 chosen for SIM analysis.

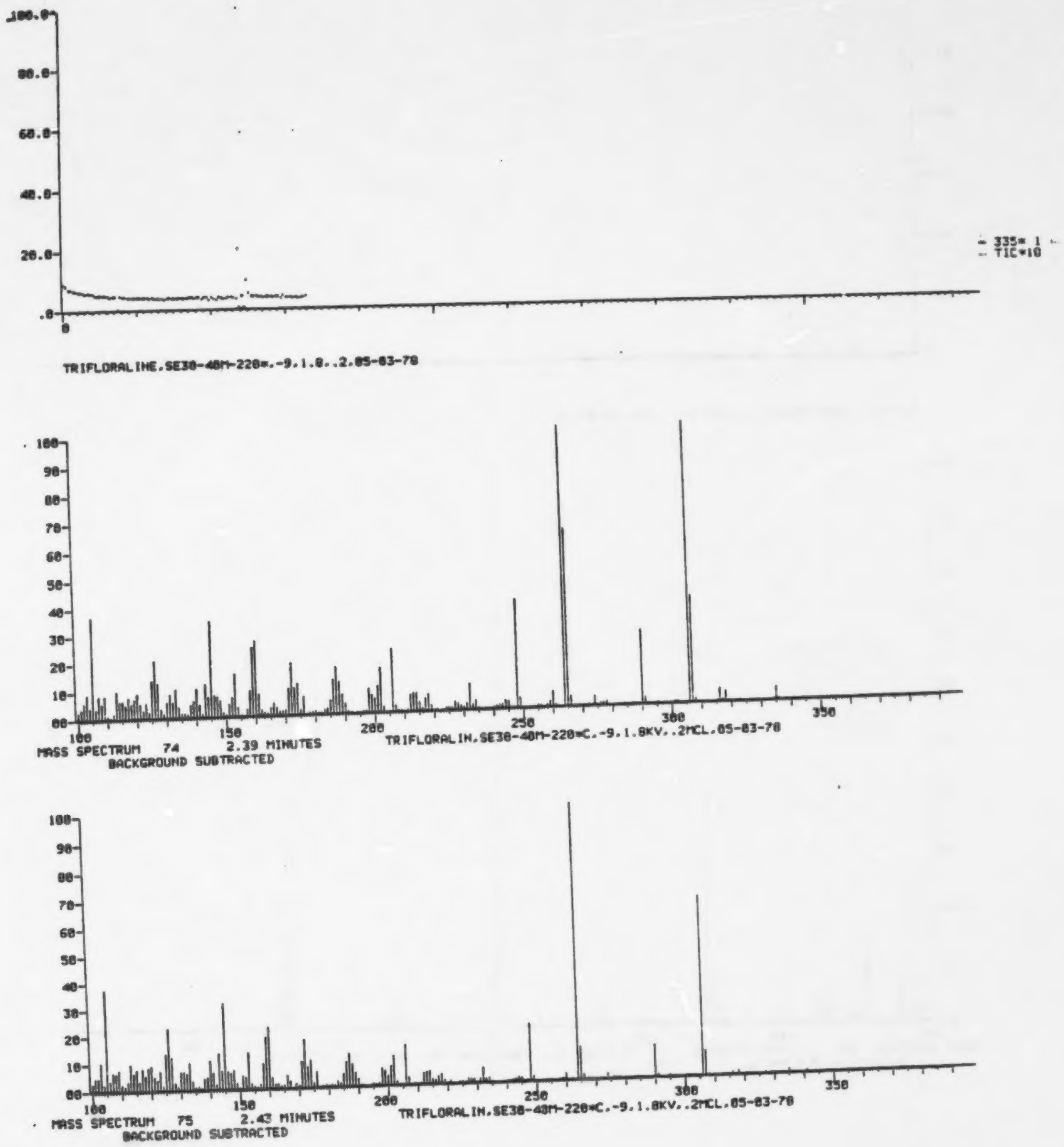


Figure L-20. Analysis of trifluralin (40 m SCOT capillary, SE-30); m/z 306 chosen for SIM analysis.

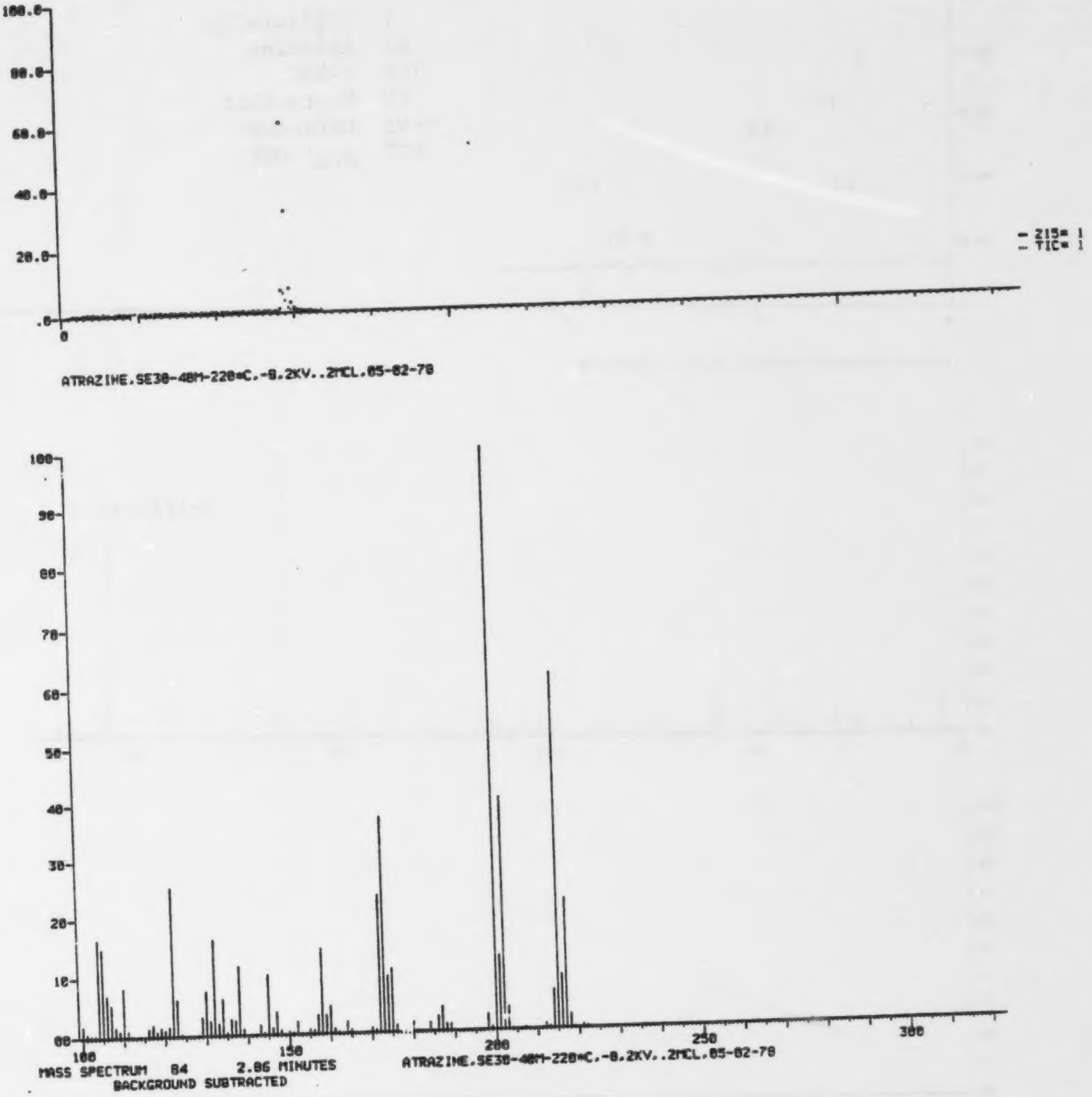


Figure L-21. Analysis of trazine (40 m SCOT capillary, SE-30); m/z 215 chosen for SIM analysis.

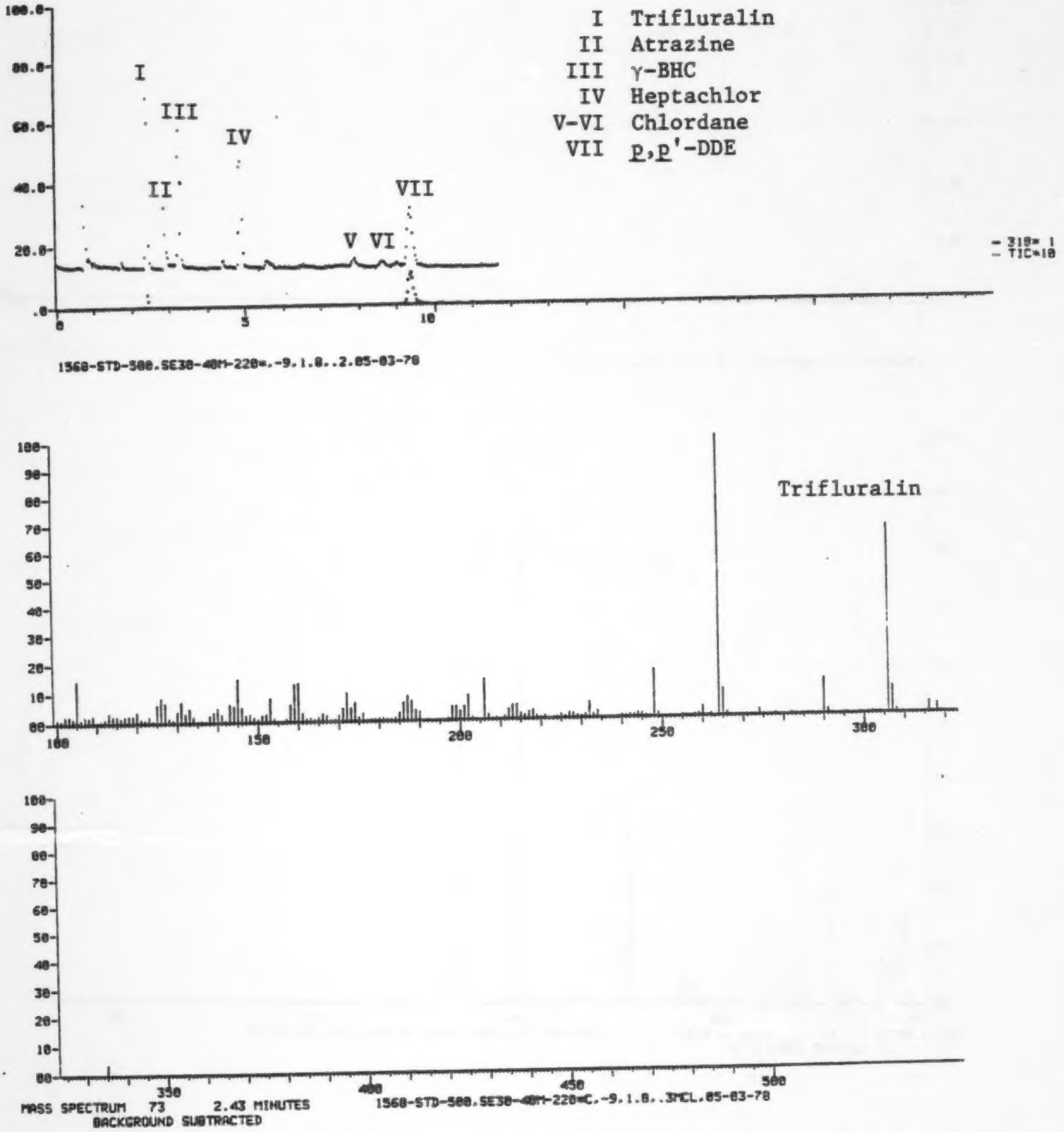


Figure L-22. Analysis of pesticide mixture, full scan mode; ∿500 ng.

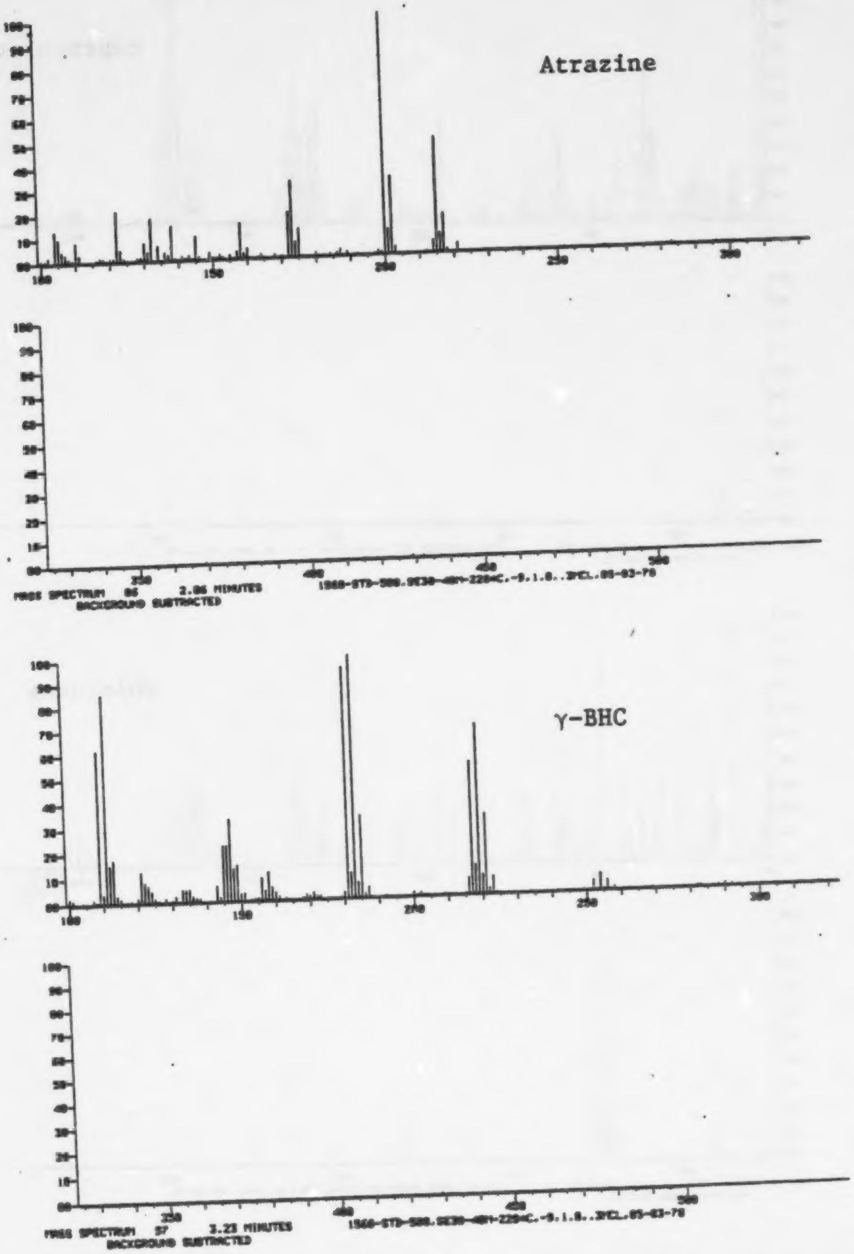


Figure L-23. Analysis of pesticide mixture, full scan mode; ∿500 ng.

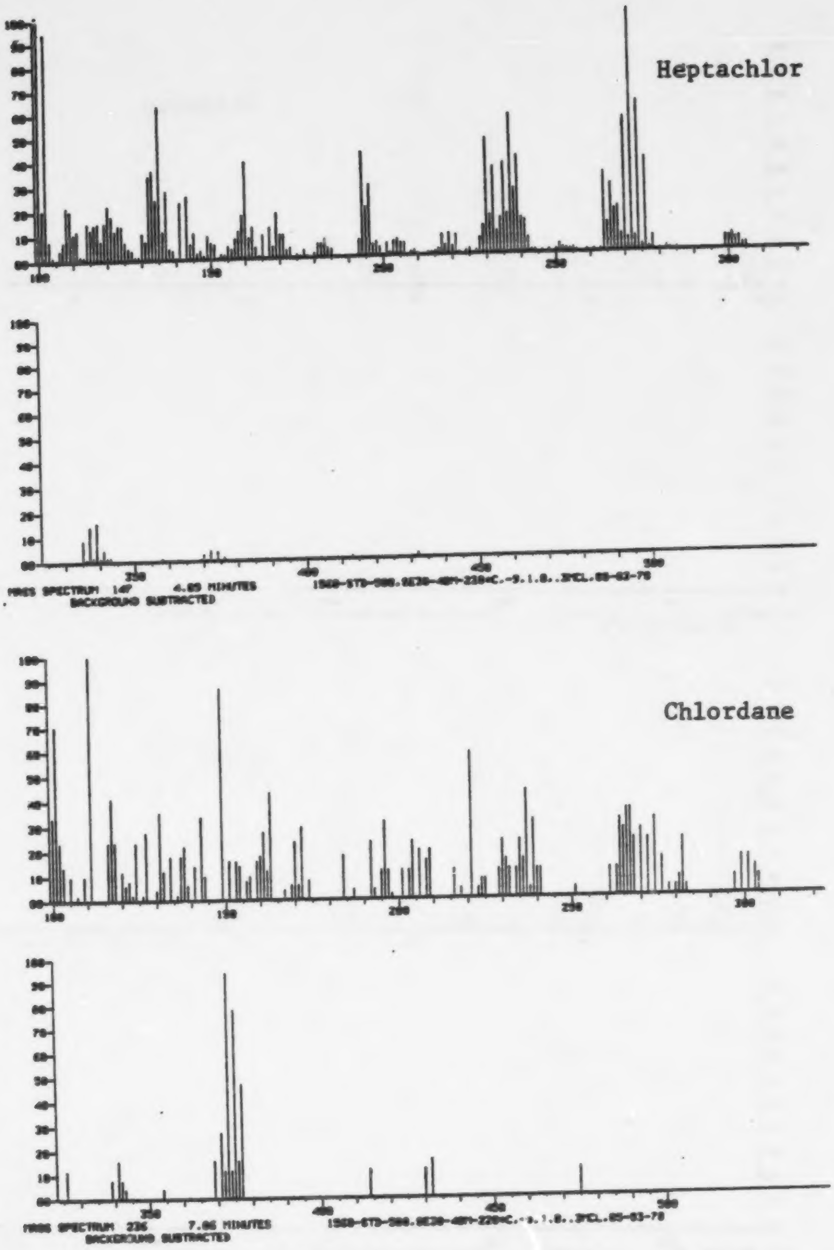


Figure L-24. Analysis of pesticide mixture, full scan mode; ∿500 ng.

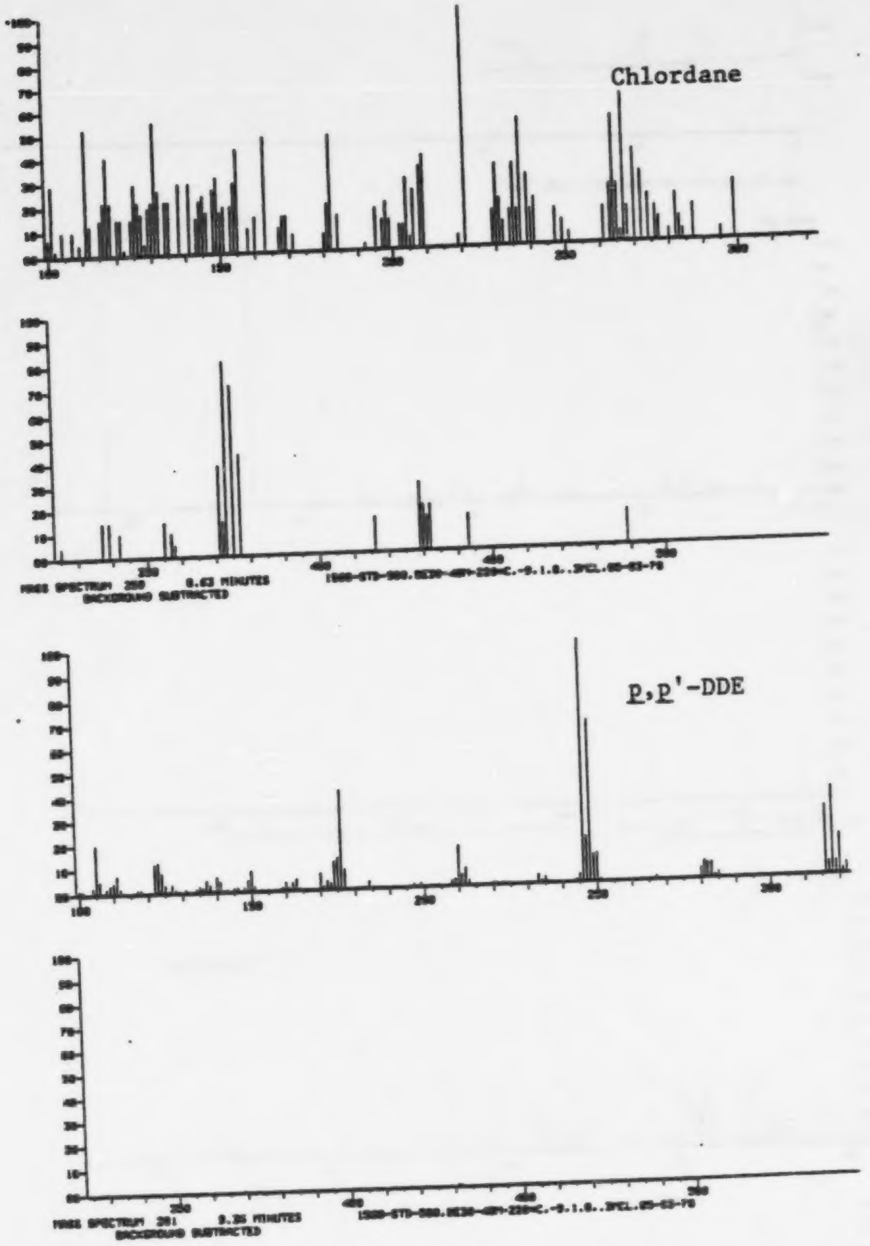


Figure L-25. Analysis of pesticide mixture, full scan mode; ∿500 ng.

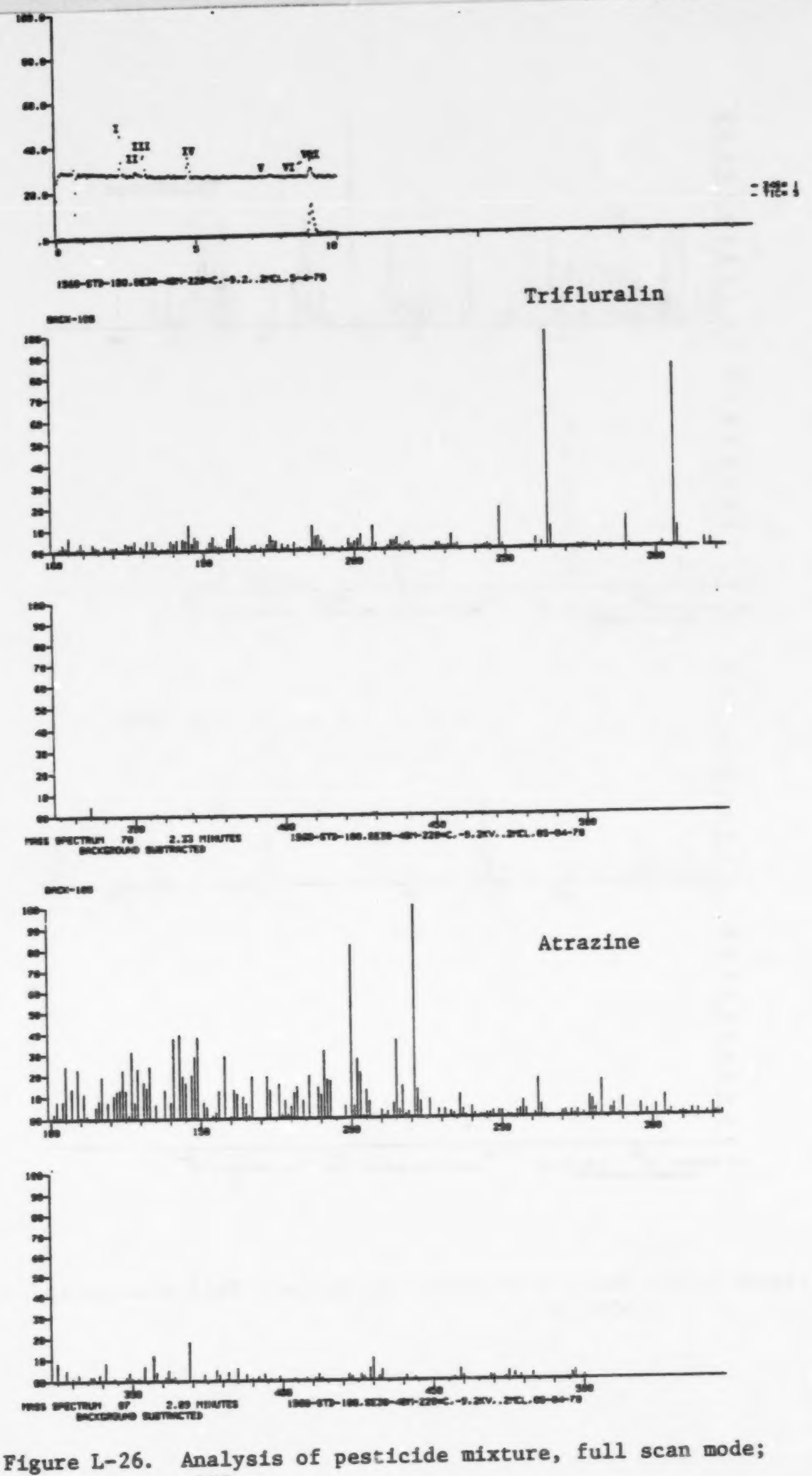


Figure L-26. Analysis of pesticide mixture, full scan mode; ∿100 ng.

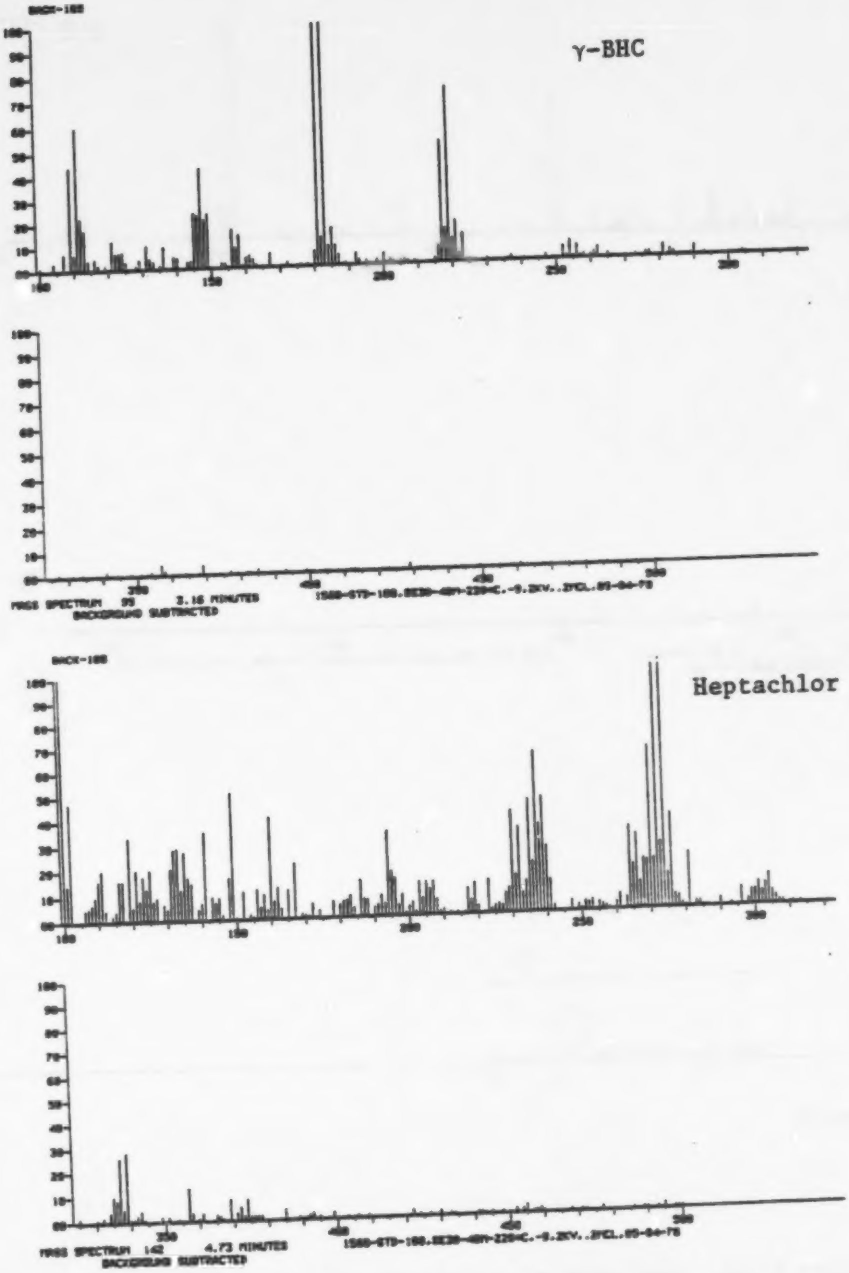


Figure L-27. Analysis of pesticide mixture, full scan mode; ∿100 ng.

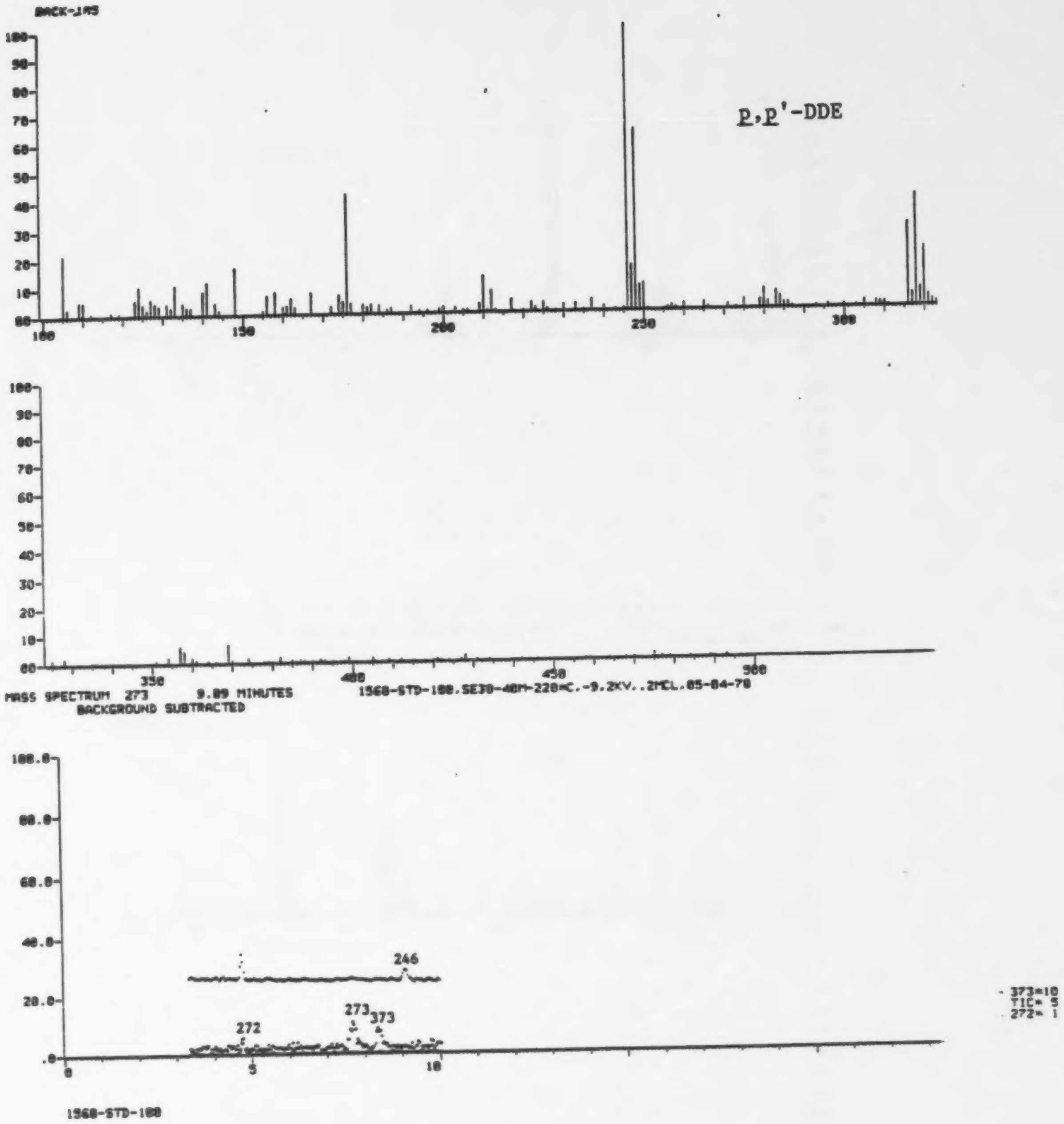


Figure L-28. Analysis of pesticide mixture, full scan mode; ∿100 ng.

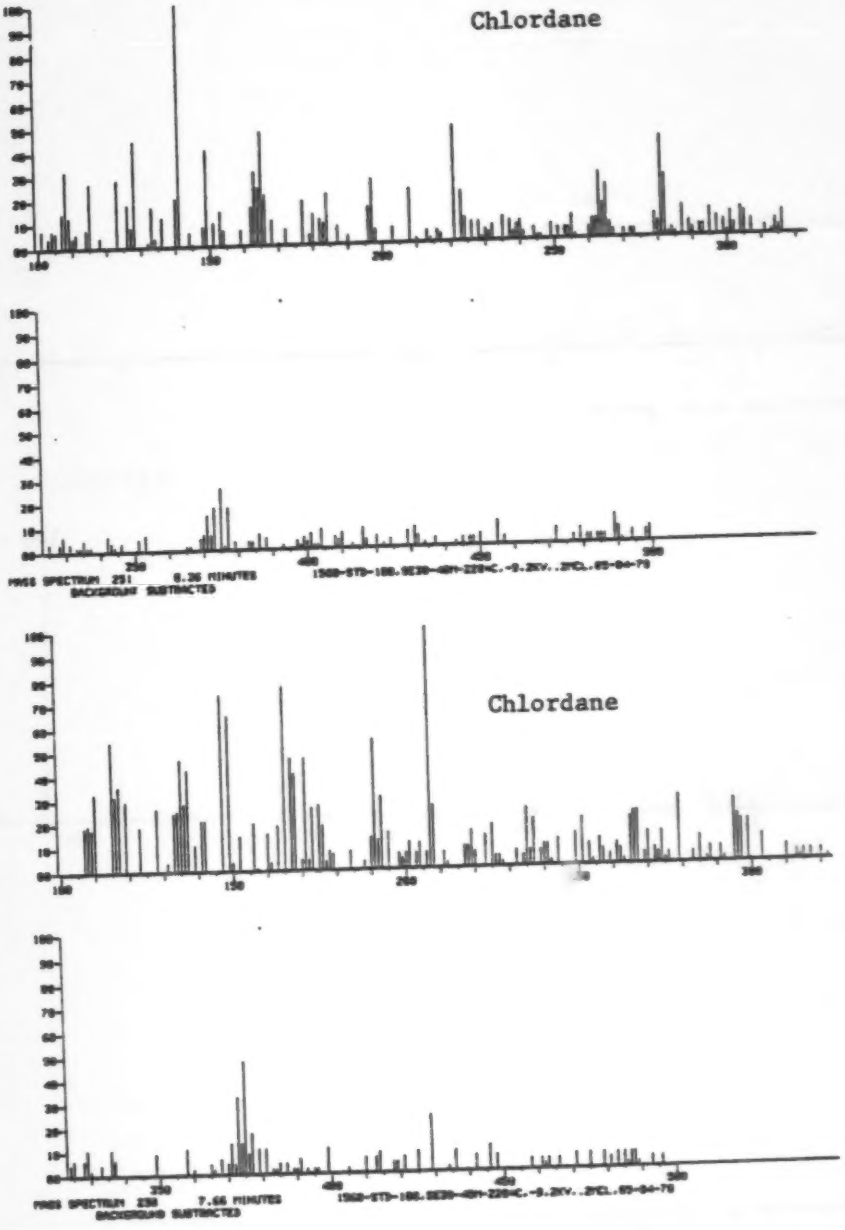


Figure L-29. Analysis of pesticide mixture, full scan mode; ∿100 ng.

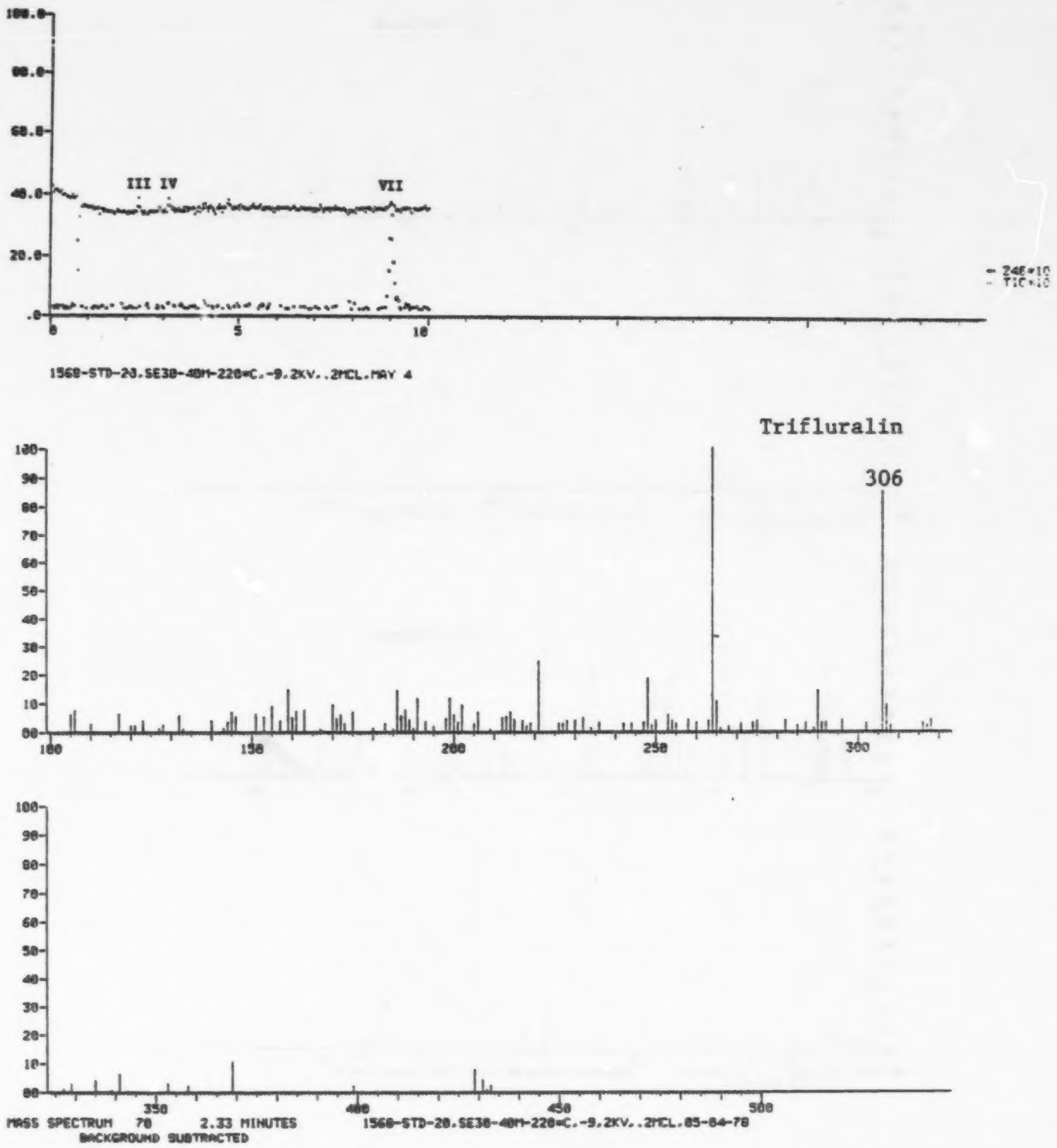


Figure L-30. Analysis of pesticide mixture, full scan mode; ∿20 ng.

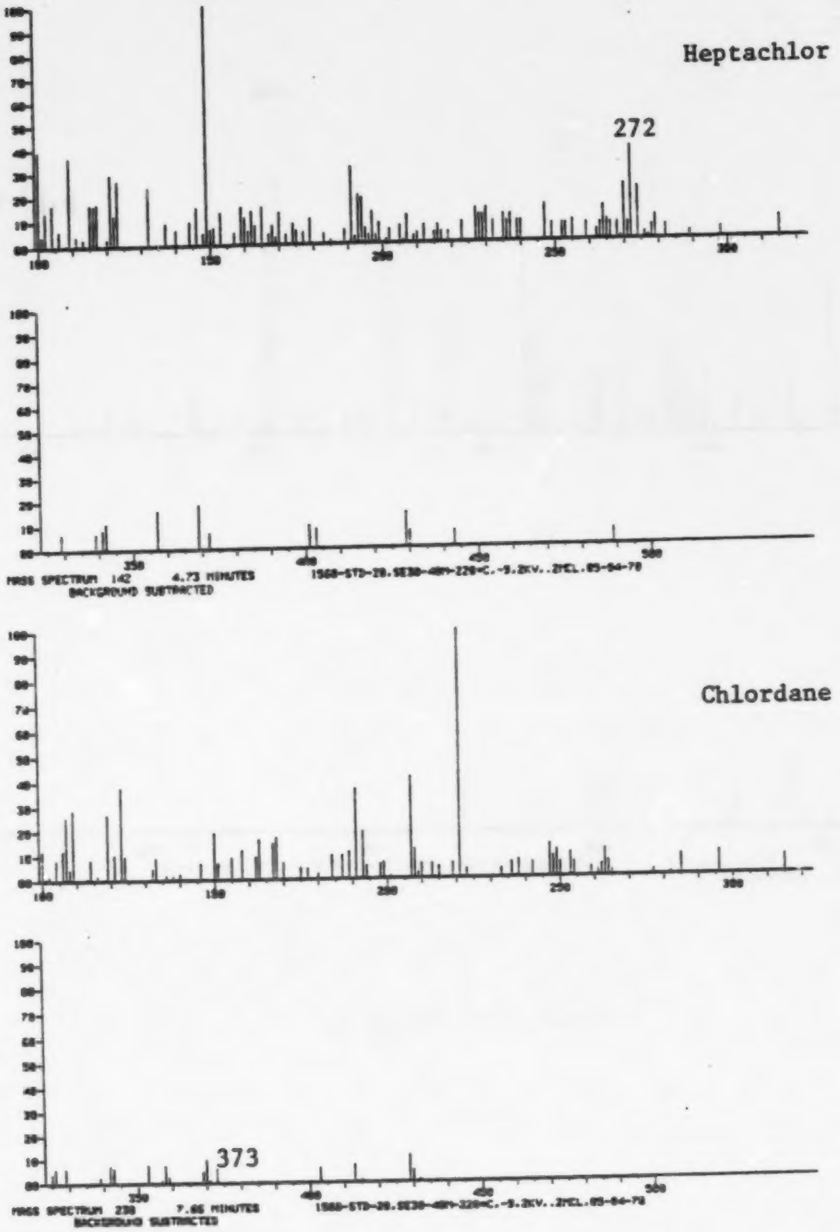


Figure L-31. Analysis of pesticide mixture, full scan mode, ∿20 ng.

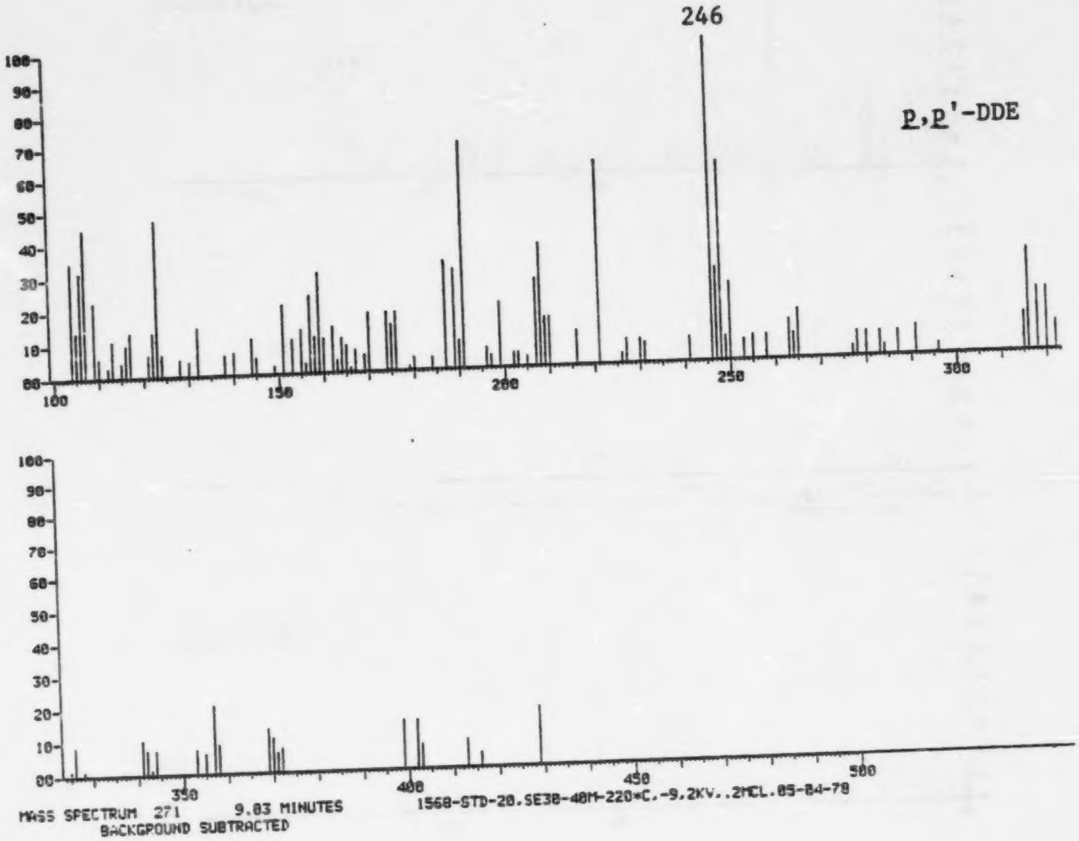


Figure L-31 (cont'd.)

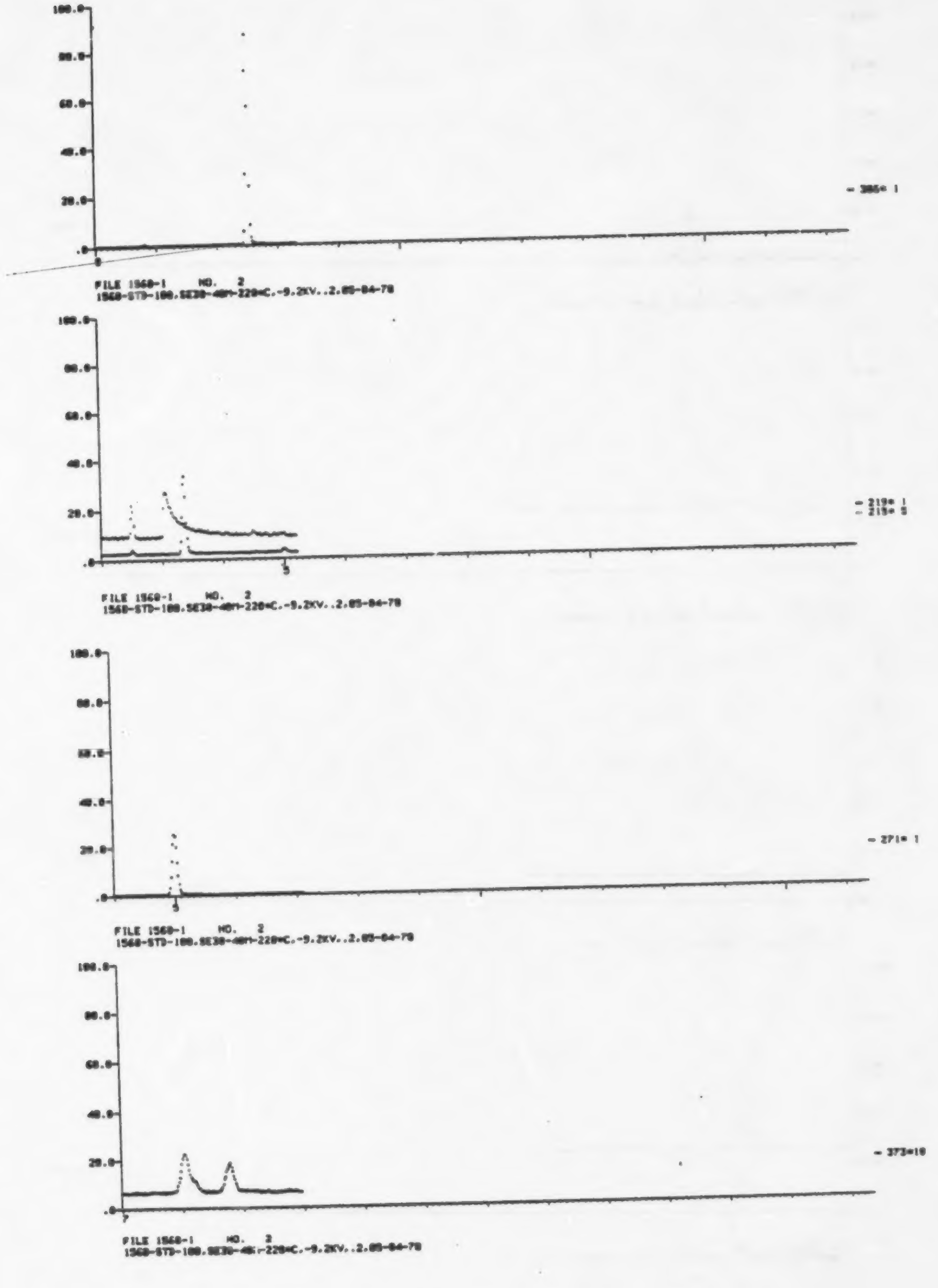


Figure L-32. Pesticide analysis, SIM mode.

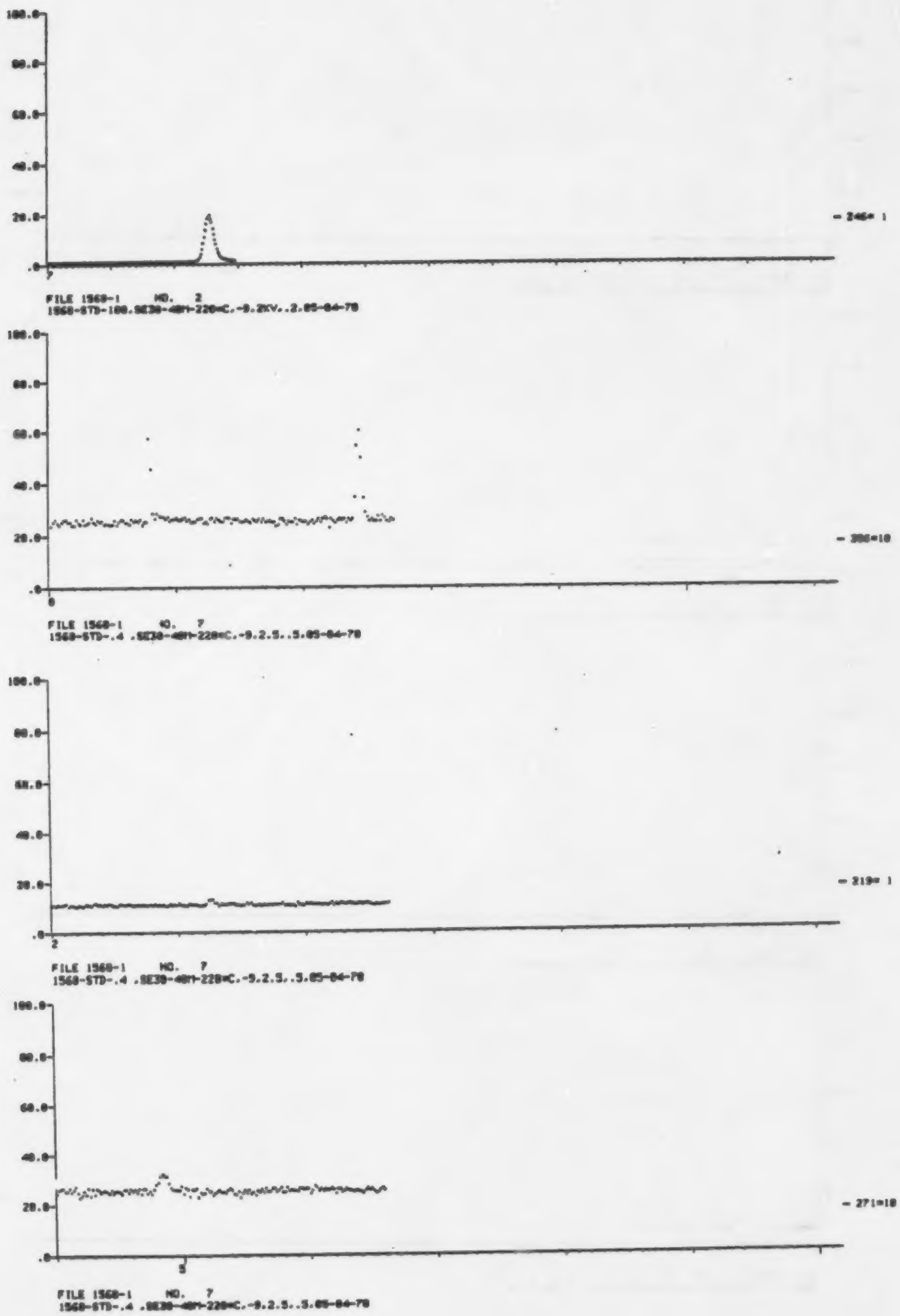


Figure L-33. Pesticide analysis, SIM mode.

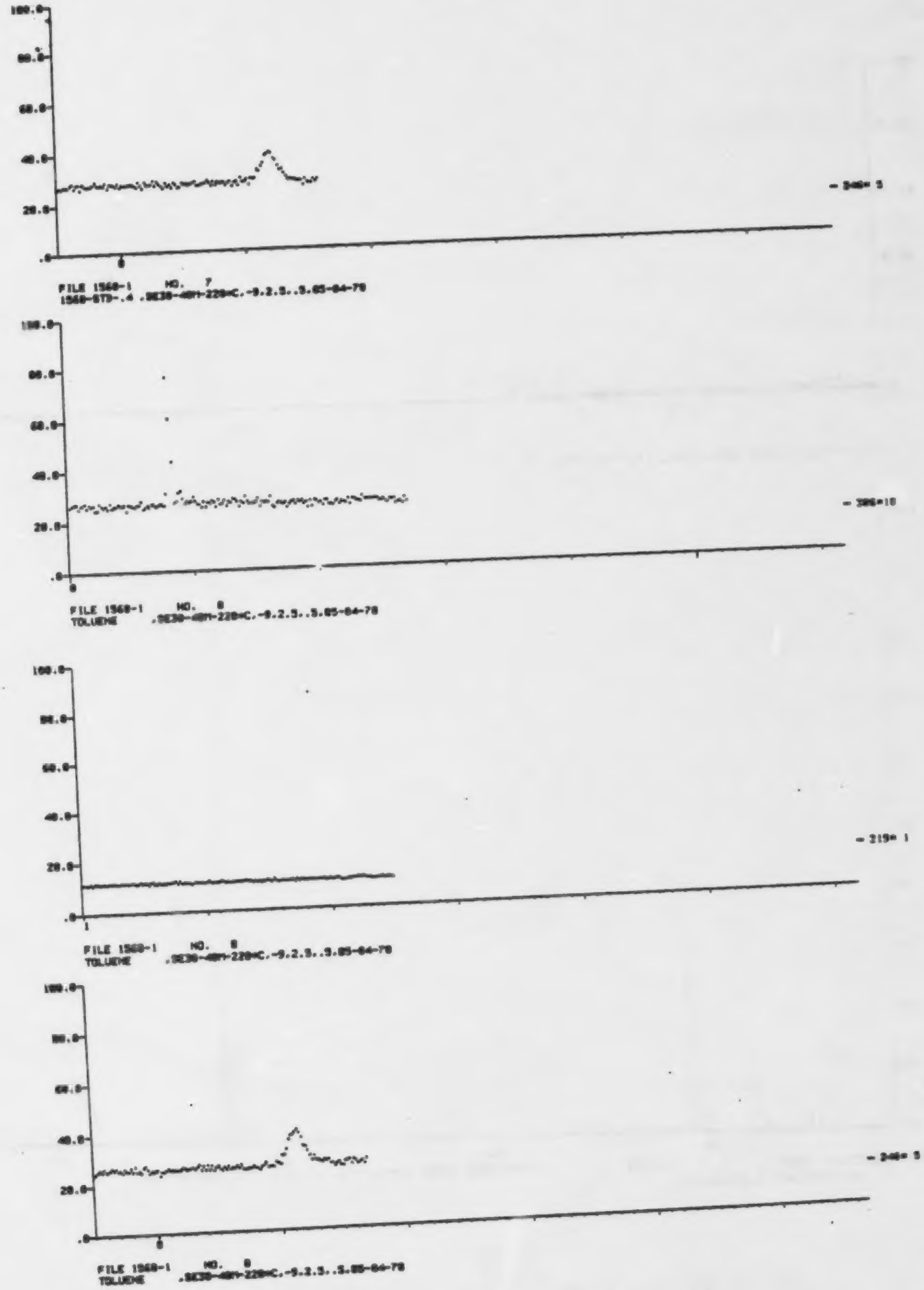


Figure L-34. Pesticide analysis, SIM mode.

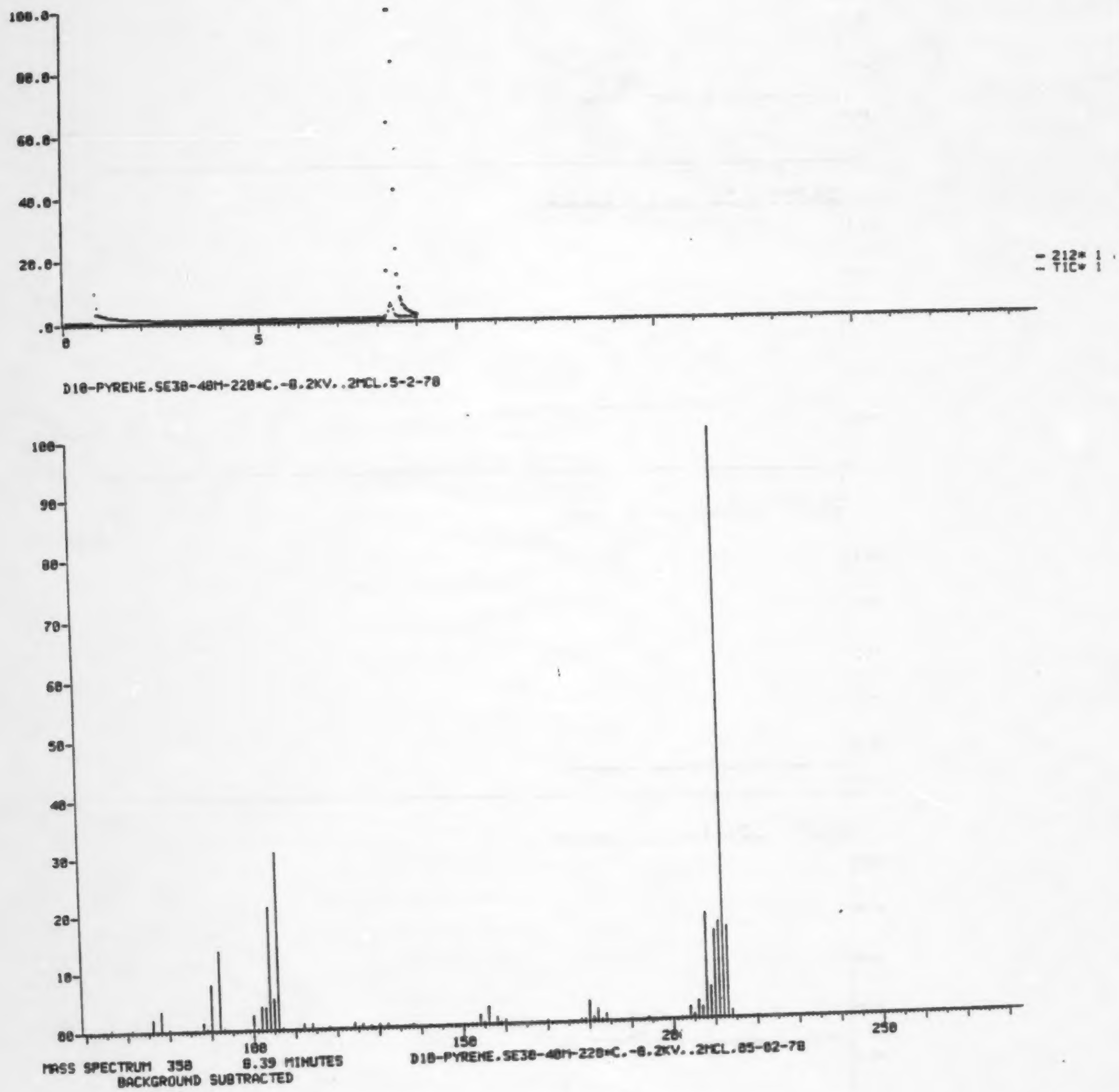


Figure L-35. Analysis of $d_{10}'$-pyrene.

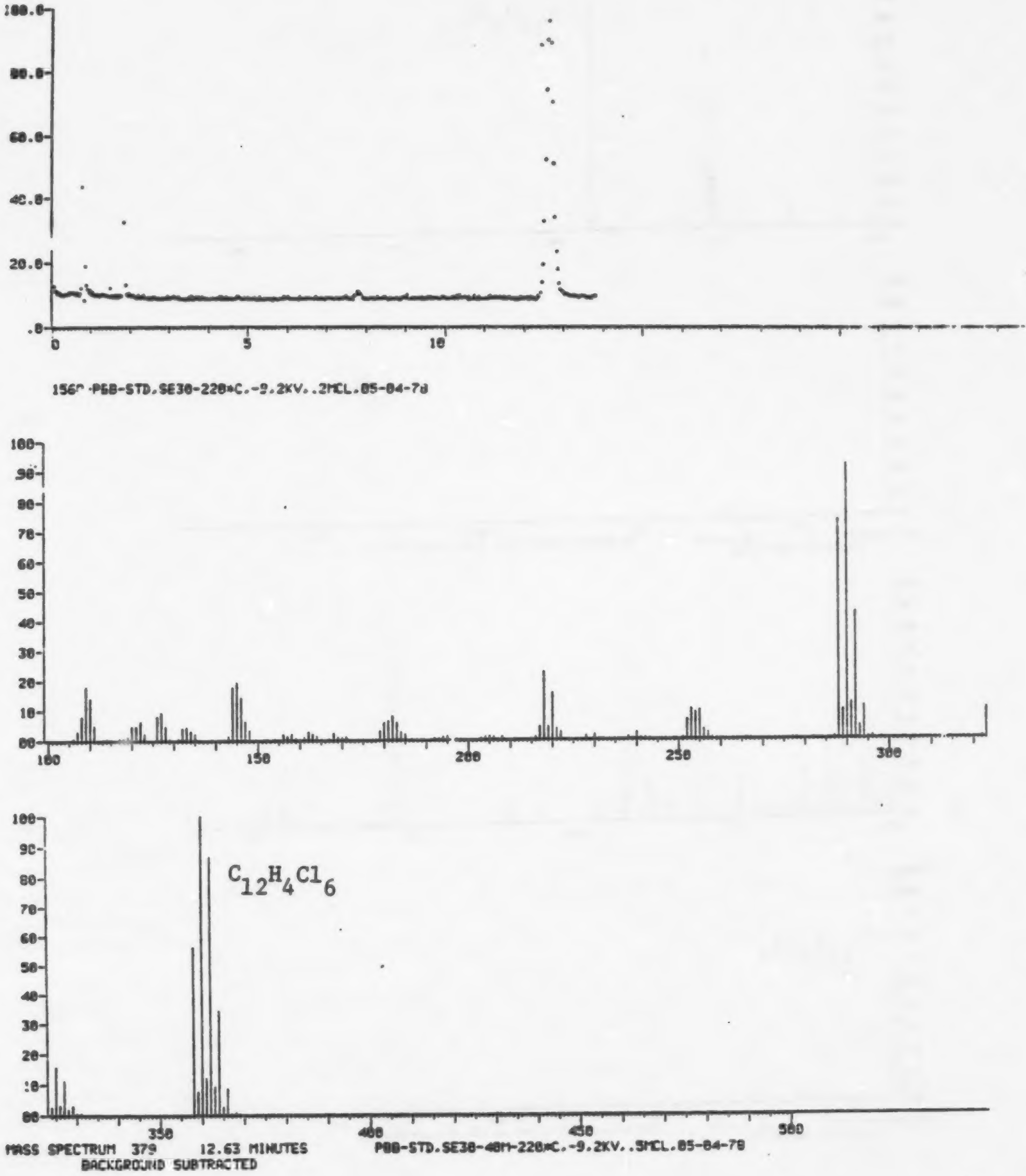


Figure L-36. Analysis of polychlorobiphenyls (40 m glass SCOT capillary, 200°C, isothermal).

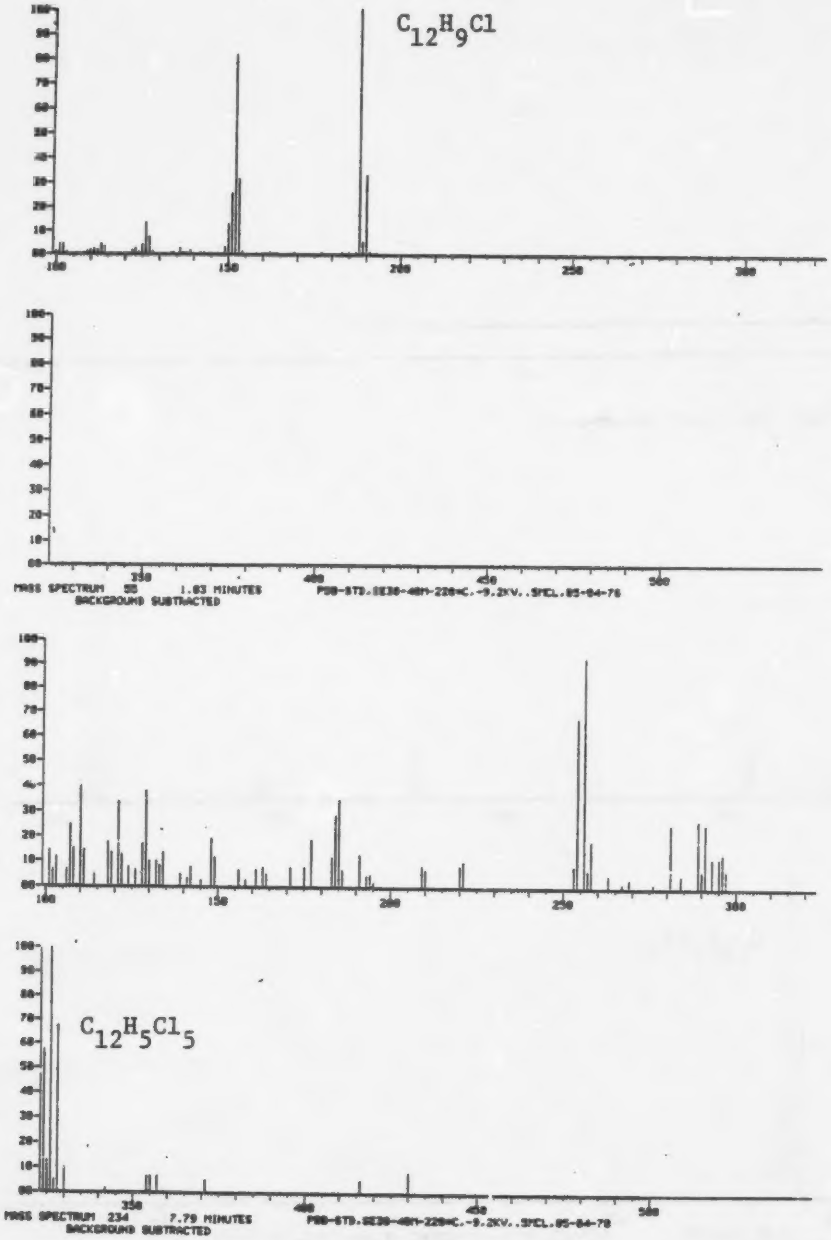


Figure L-37. Analysis of polychlorinated biphenyls (40 m glass SCOT capillary, 220°C isothermal).

Table L-1. STANDARD SOLUTIONS OF POLYCHLORINATED BIPHENYLS

| Solution | 2-Chlorobiphenyl, ng/μl | Hexachlorobiphenyl, ng/μl | Decachlorobiphenyl, ng/μl |
|---|---|---|---|
| STD-100 | 97.9 | 536 | 1088 |
| STD-20 | 20.8 | 114 | 231 |
| STD-2 | 2 | 11.4 | 23 |
| STD-0.2 | 0.2 | ∿1 | ∿2.3 |
| STD-0.04 | 0.04 | ∿0.2 | ∿0.45 |

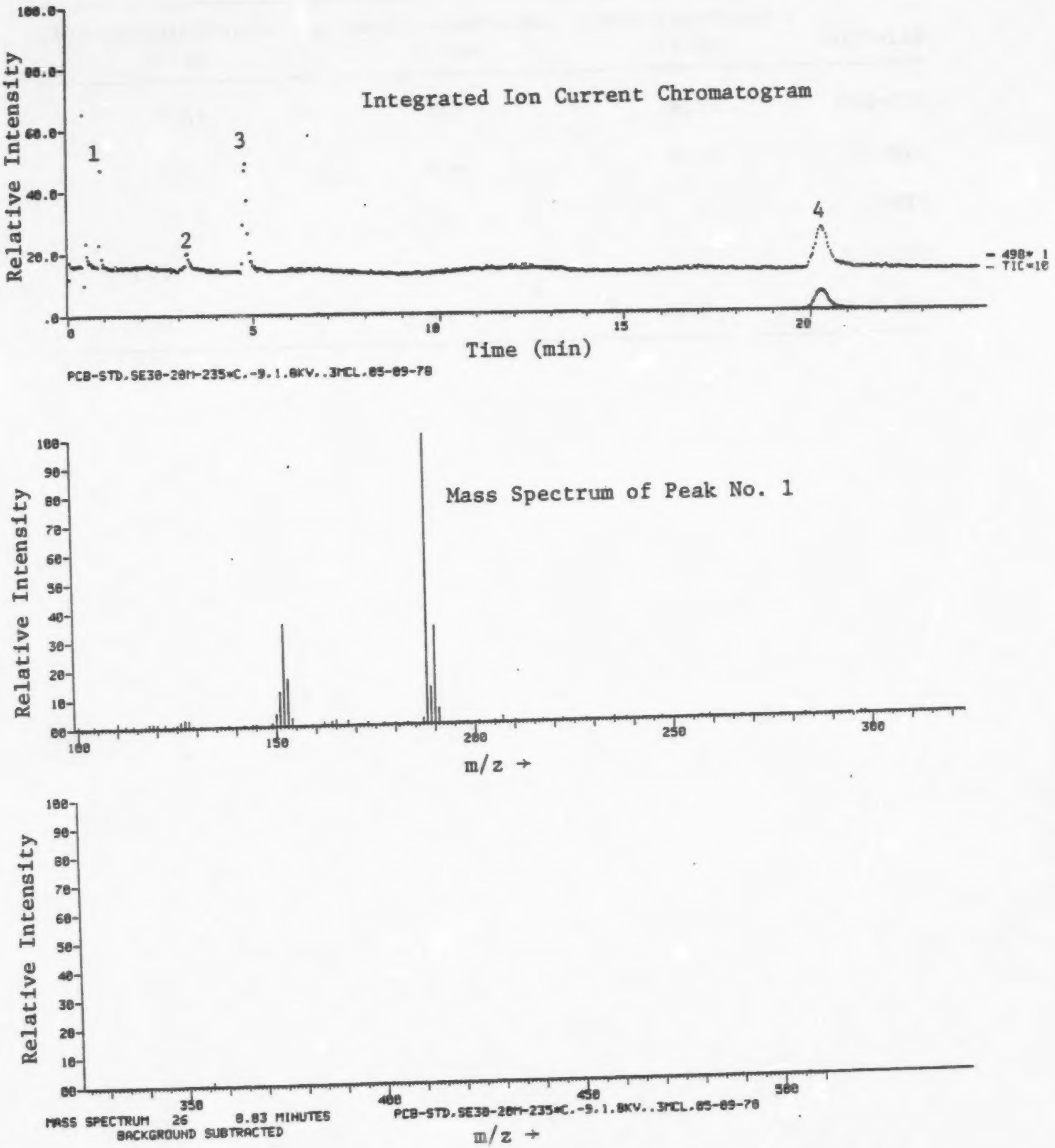


Figure L-38. Analysis of PCB mixture (SID-100).

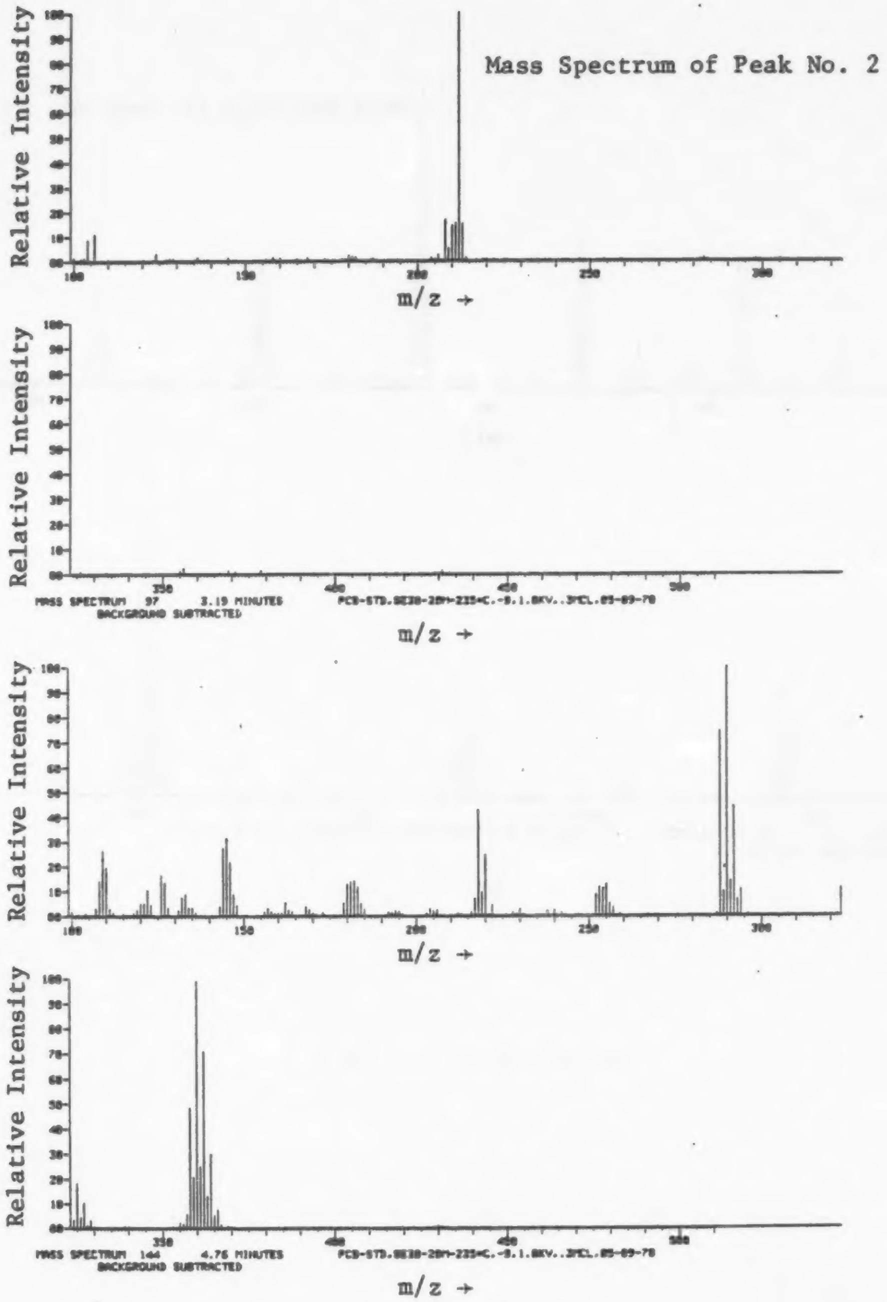


Figure L-38 (cont'd.)

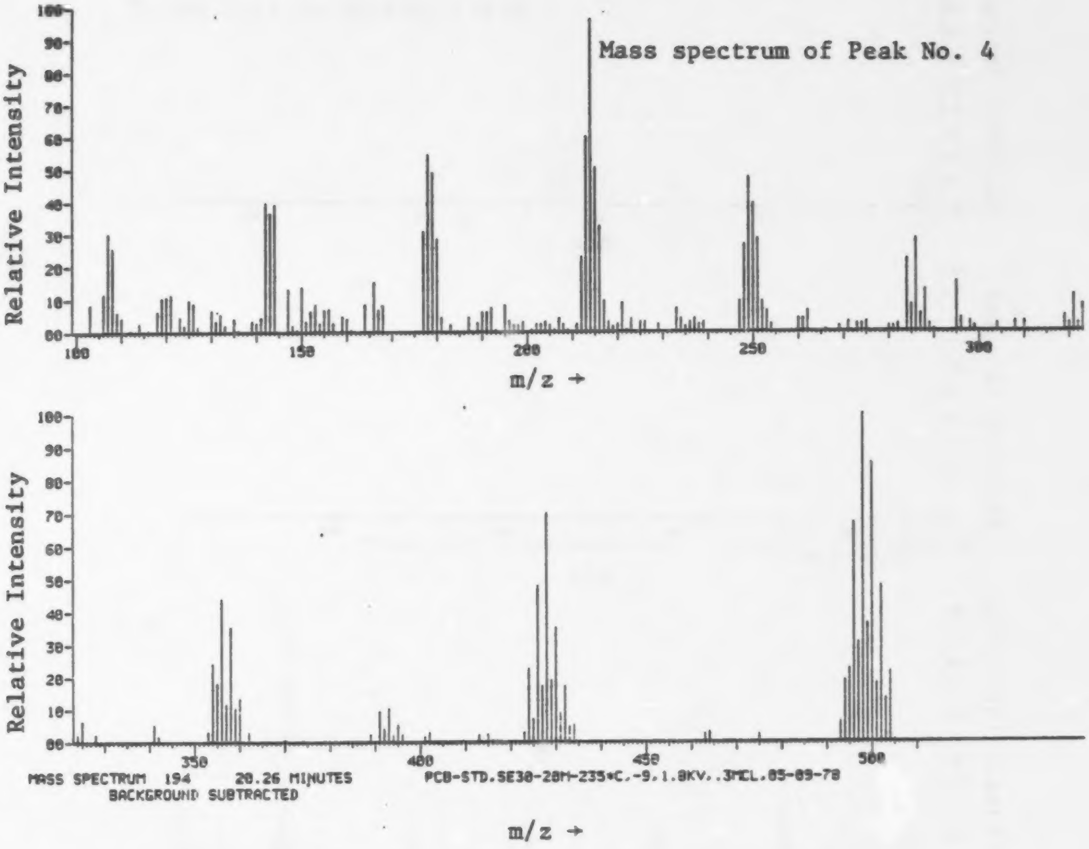


Figure L-38 (cont'd.)

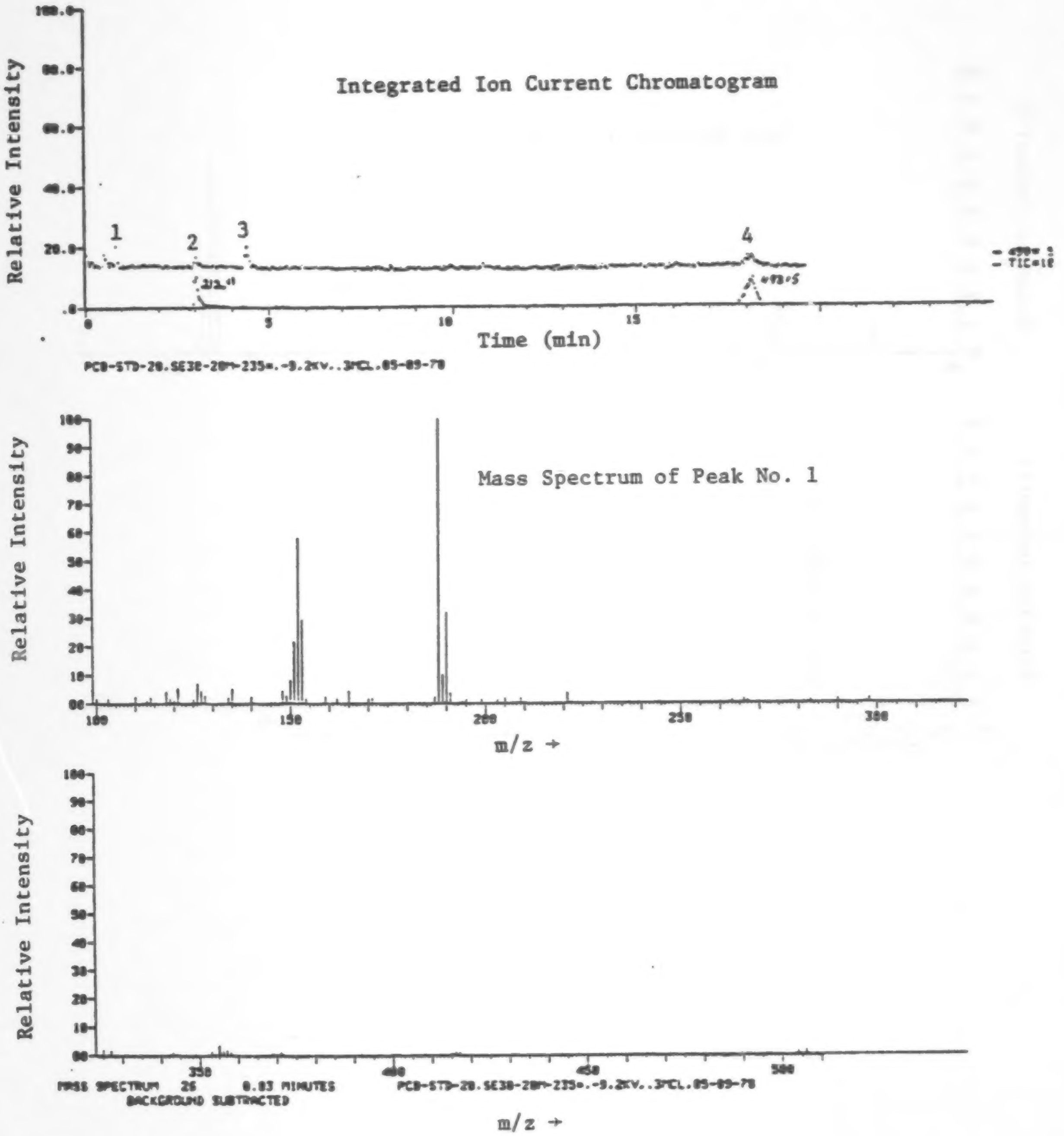


Figure L-39. Analysis of PCB Standard Mixture (STD-20)

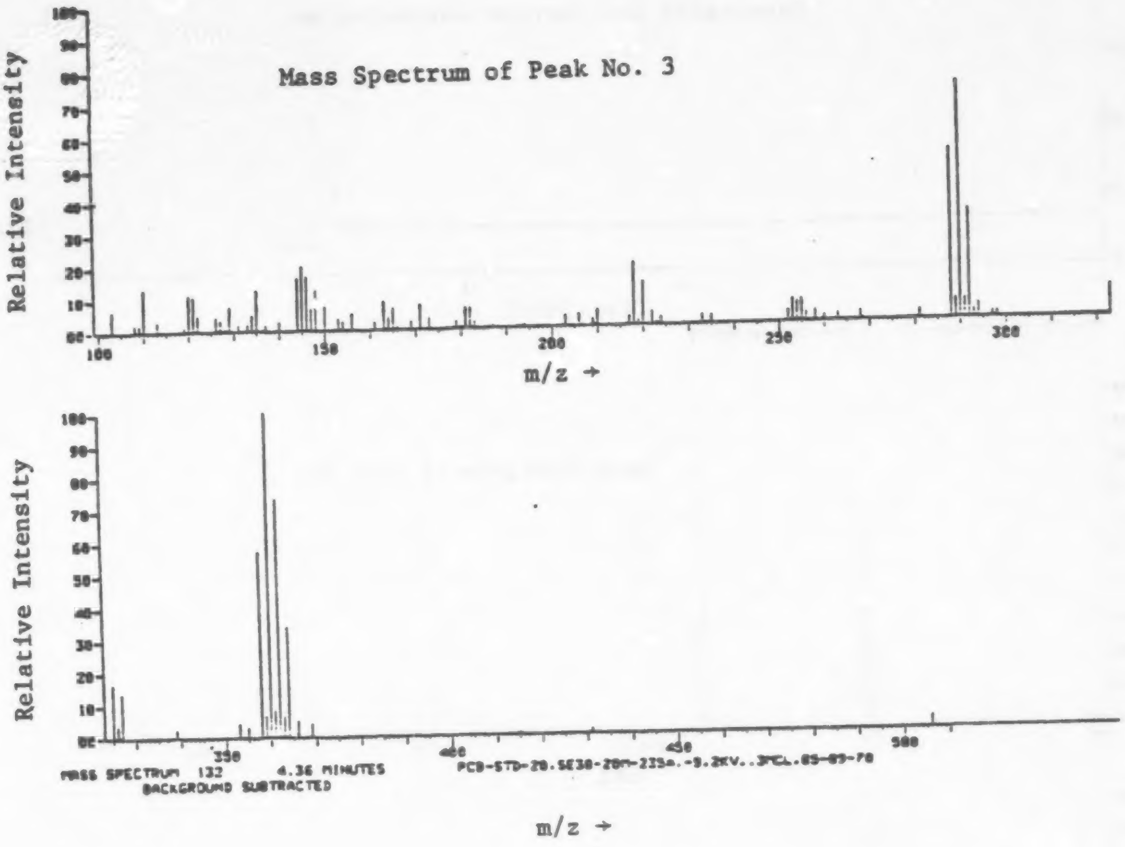
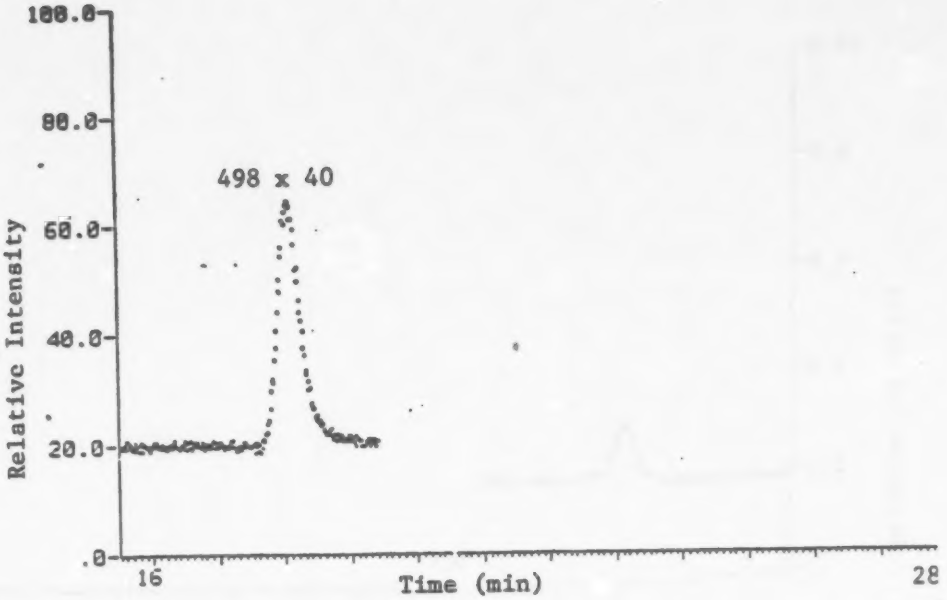
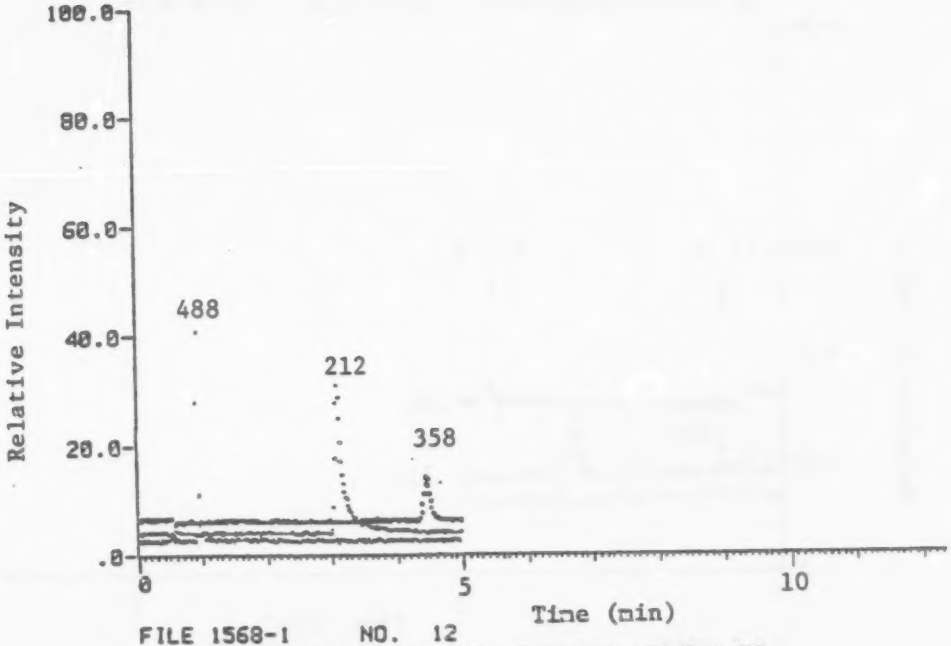


Figure L-39 (cont'd.)

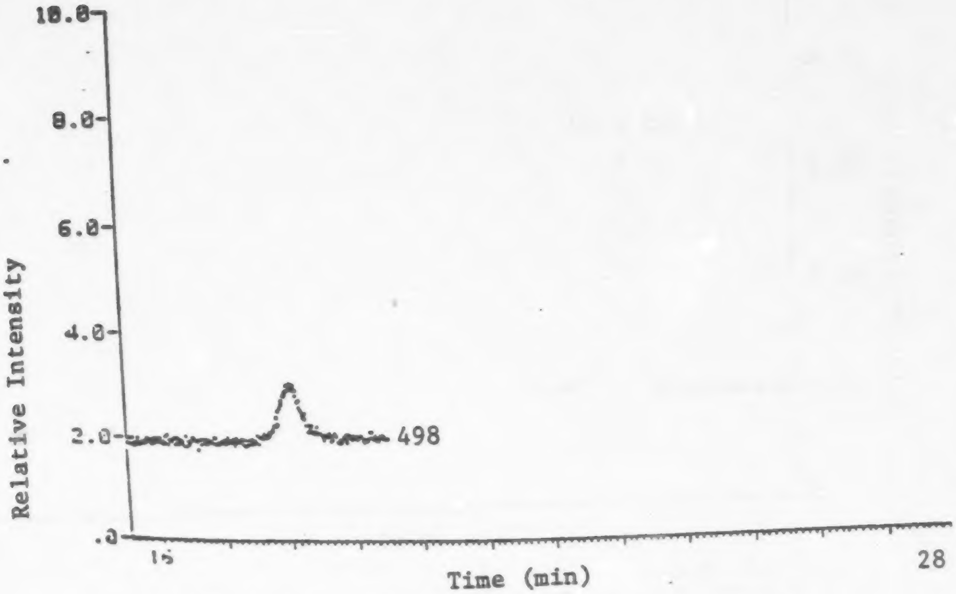


FILE 1568-1 NO. 11
PCB-STD-2 .SE30-20M-235*C.-9.2.5KV..3.85-09-78

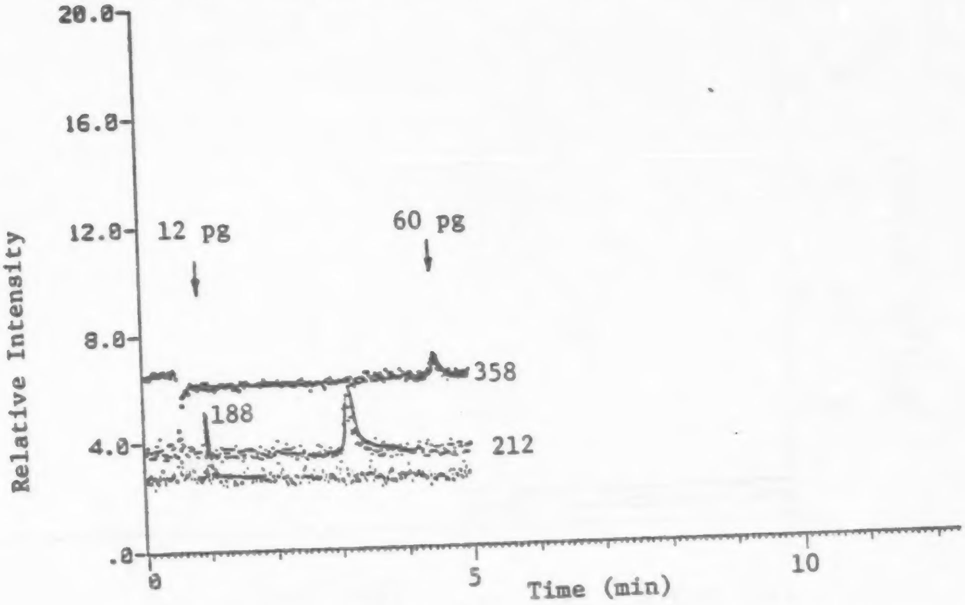


FILE 1568-1 NO. 12
PCB-STD-2 .SE30-20M-235*C.-9.2.5KV..15.5-9-78

Figure L-40. Representative Output from Analysis of PCB Mixtures, Selected Ion Monitoring Mode

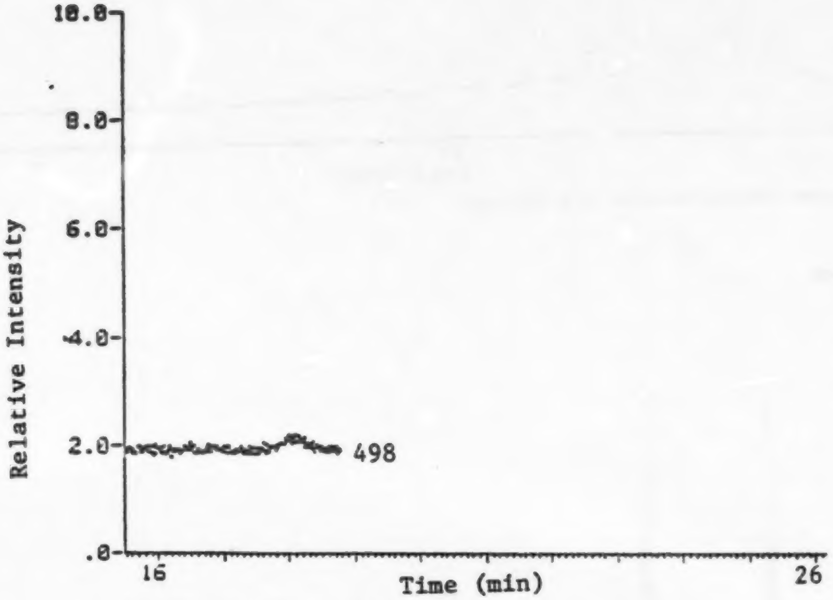


File 1568-1 NO. 13
PCB-STD-.2.SE30-20M-235*C.-9.2.5KV.,30.5-9-78



FILE 1568-1 NO. 14
PCB-STD.04.SE30-20M-235*C.-9.2.5KV..30.5-9-78

Figure L-40 (cont'd.)



FILE 1568-1 NO. 17
PCB-STD.04.SE30-20M-235°C.-9.2.5KV..30.5-9-78

Figure L-40 (cont'd.)

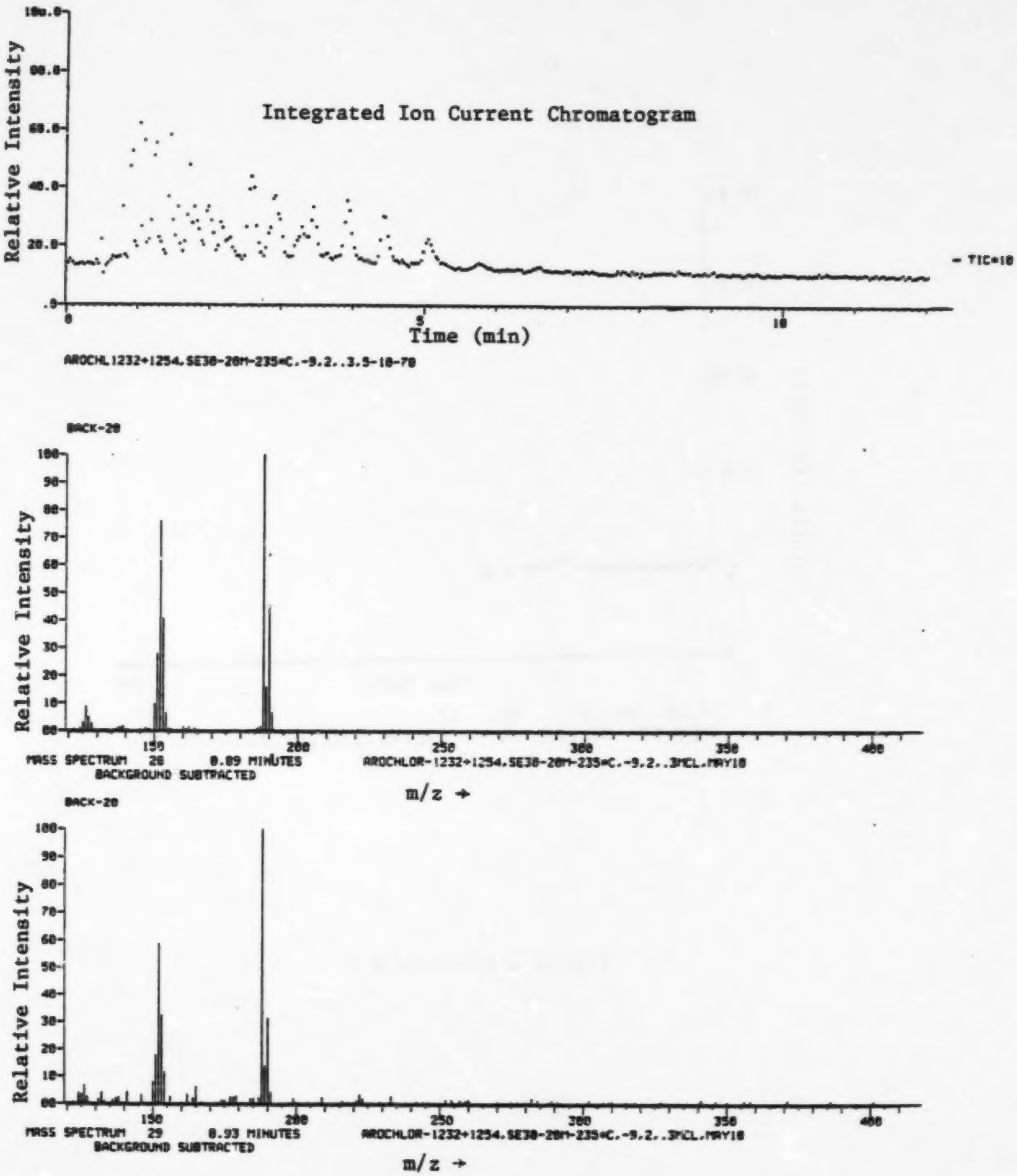


Figure L-41. Analysis of arochlor mixture, full scan mode.

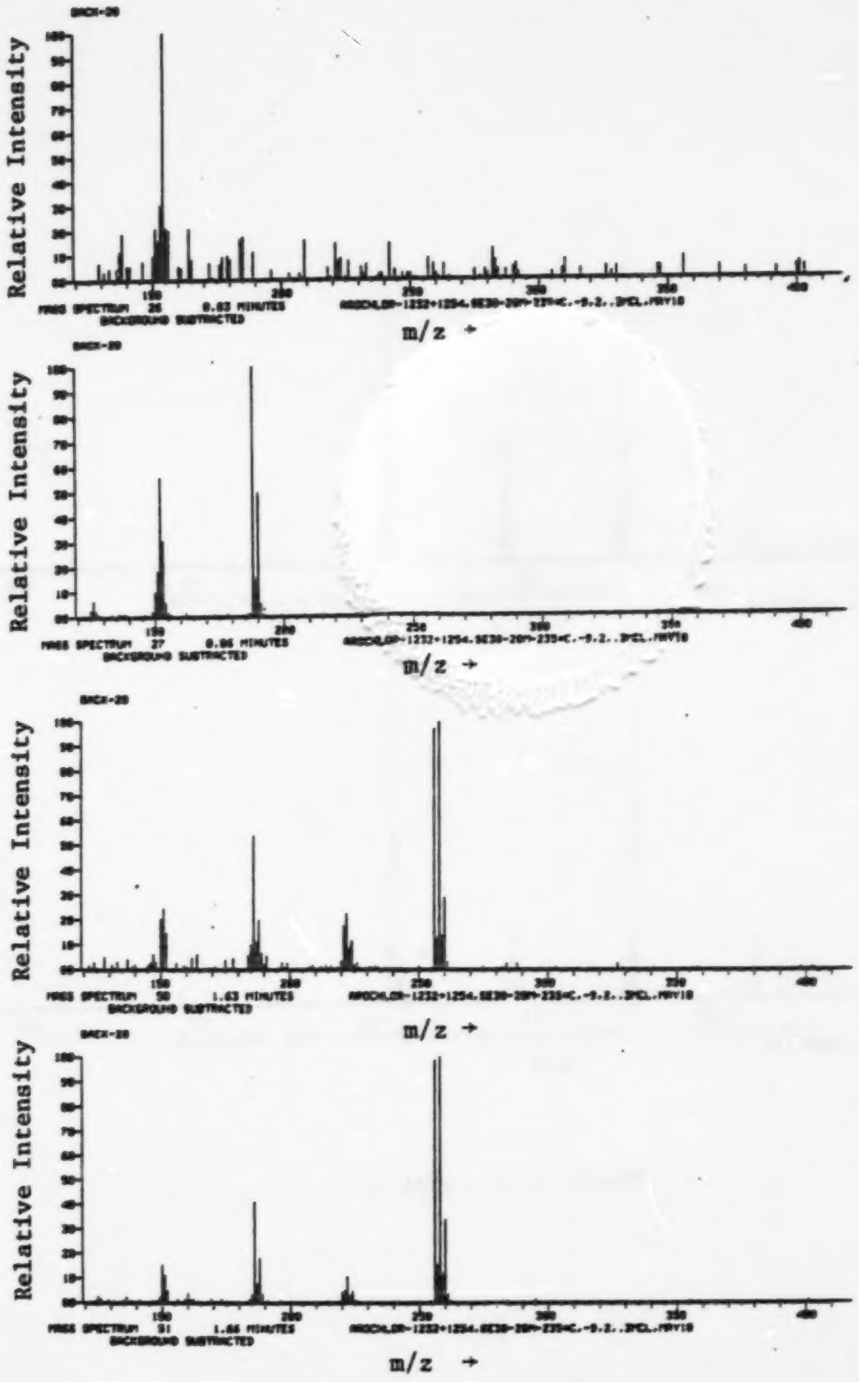


Figure L-41 (cont'd.)

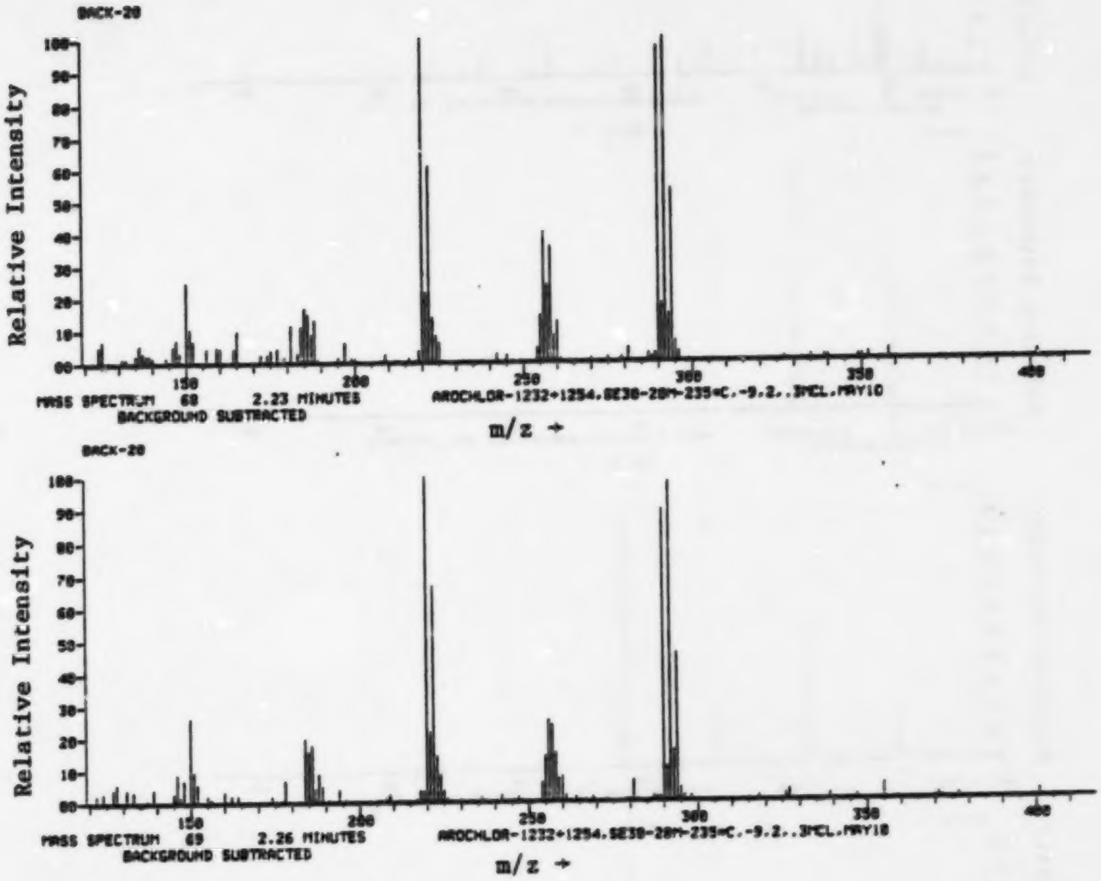


Figure L-41 (cont'd.)

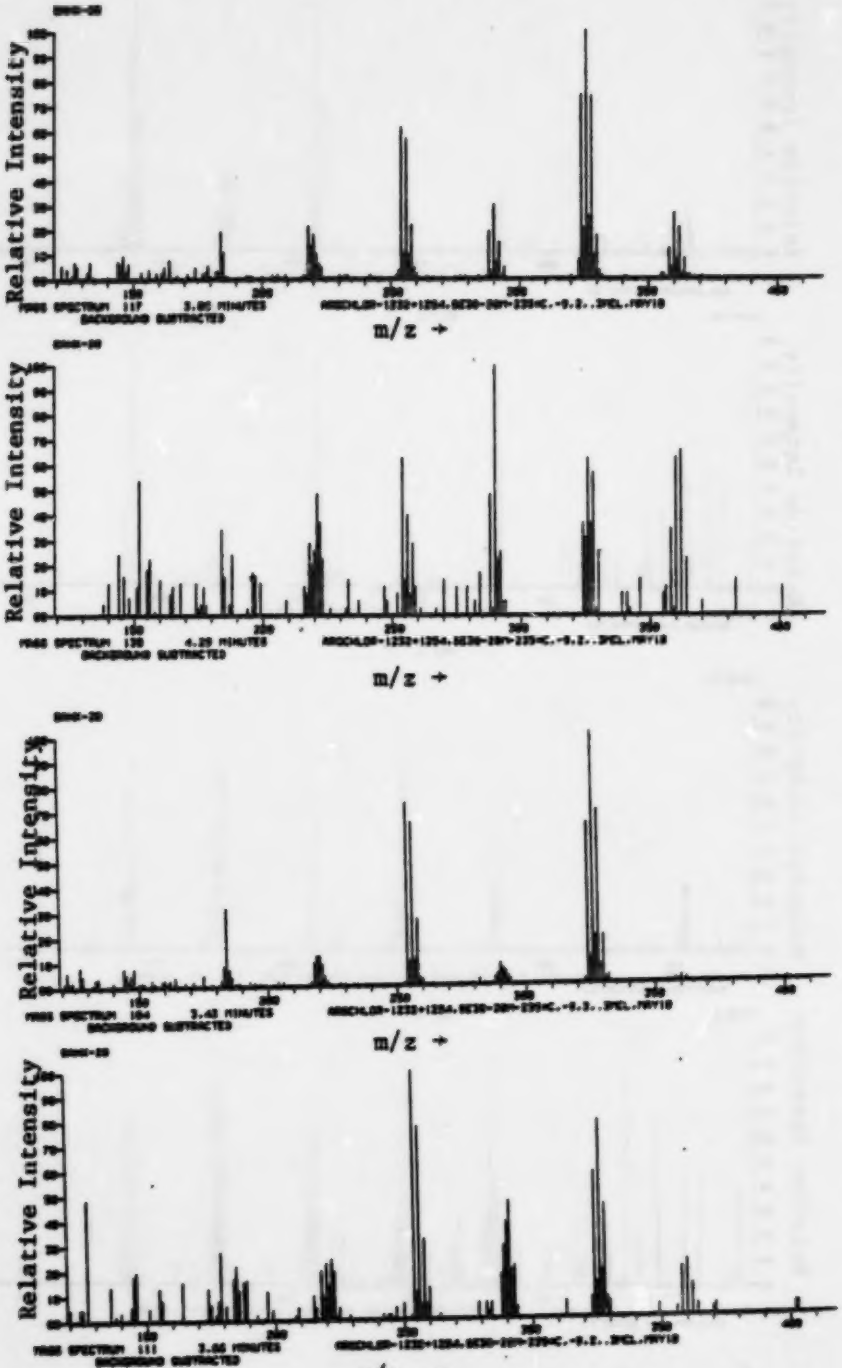


Figure L-41 (cont'd.)

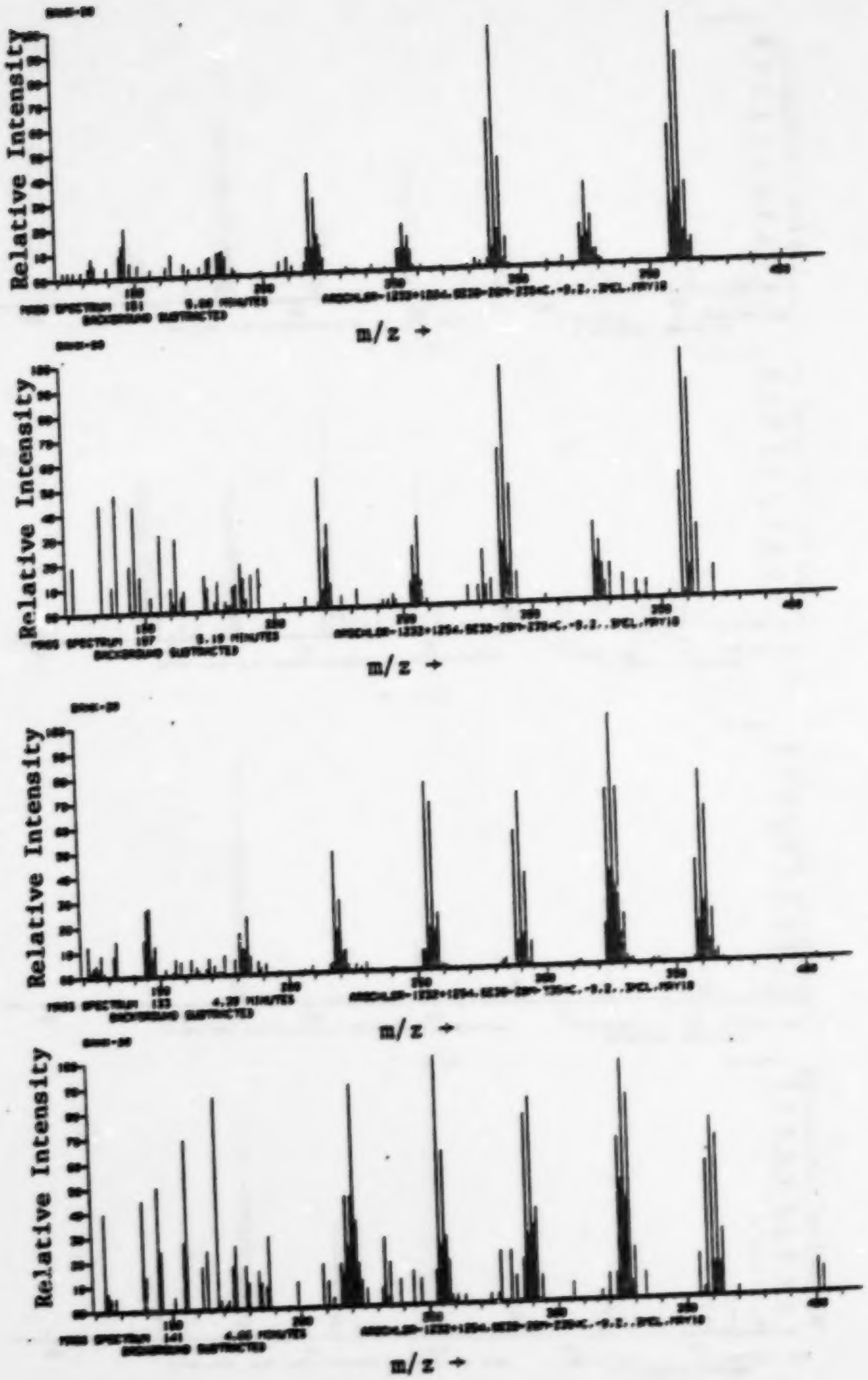


Figure L-41 (cont'd.)

## APPENDIX M

## GC/MS/COMP SINGLE ION PLOTS (SIP) OF EXTRACTABLE HALOGENATED ORGANICS USED IN RMR DETERMINATIONS

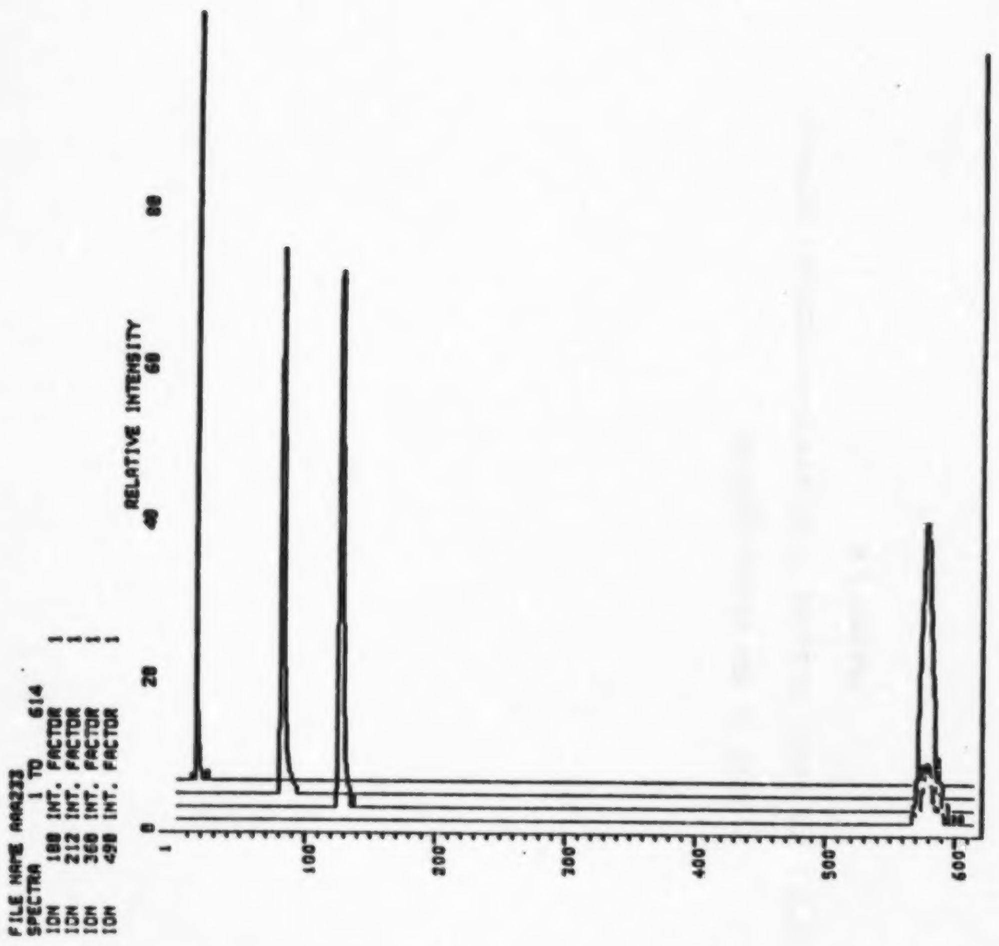


Figure M-1. SIP for PCB Mix: 104 ng/µl of 2-chlorobiphenyl, $\underline{m}/\underline{z}$ 188; 570 ng/µl hexachlorobiphenyl, $\underline{m}/\underline{z}$ 360; 1156 ng/µl decachlorobiphenyl, $\underline{m}/\underline{z}$ 498.

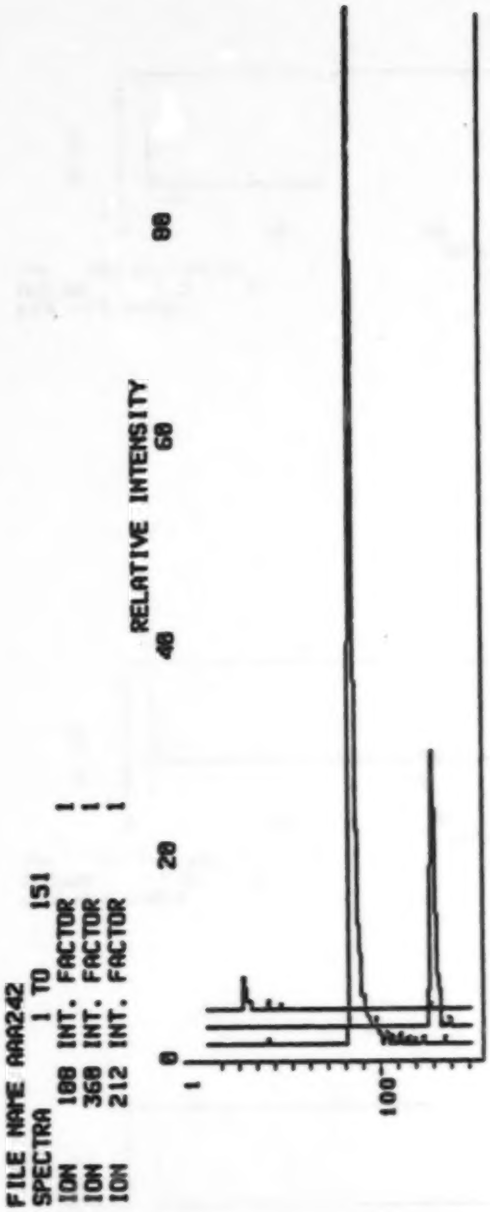


Figure M-2. SIP for PCB mix - 10.4 ng/μl of hexachlorobiphenyl, $\underline{m}/\underline{z}$ 360; 8.4 ng-$d_{10}$-pyrene, $\underline{m}/\underline{z}$ 212.

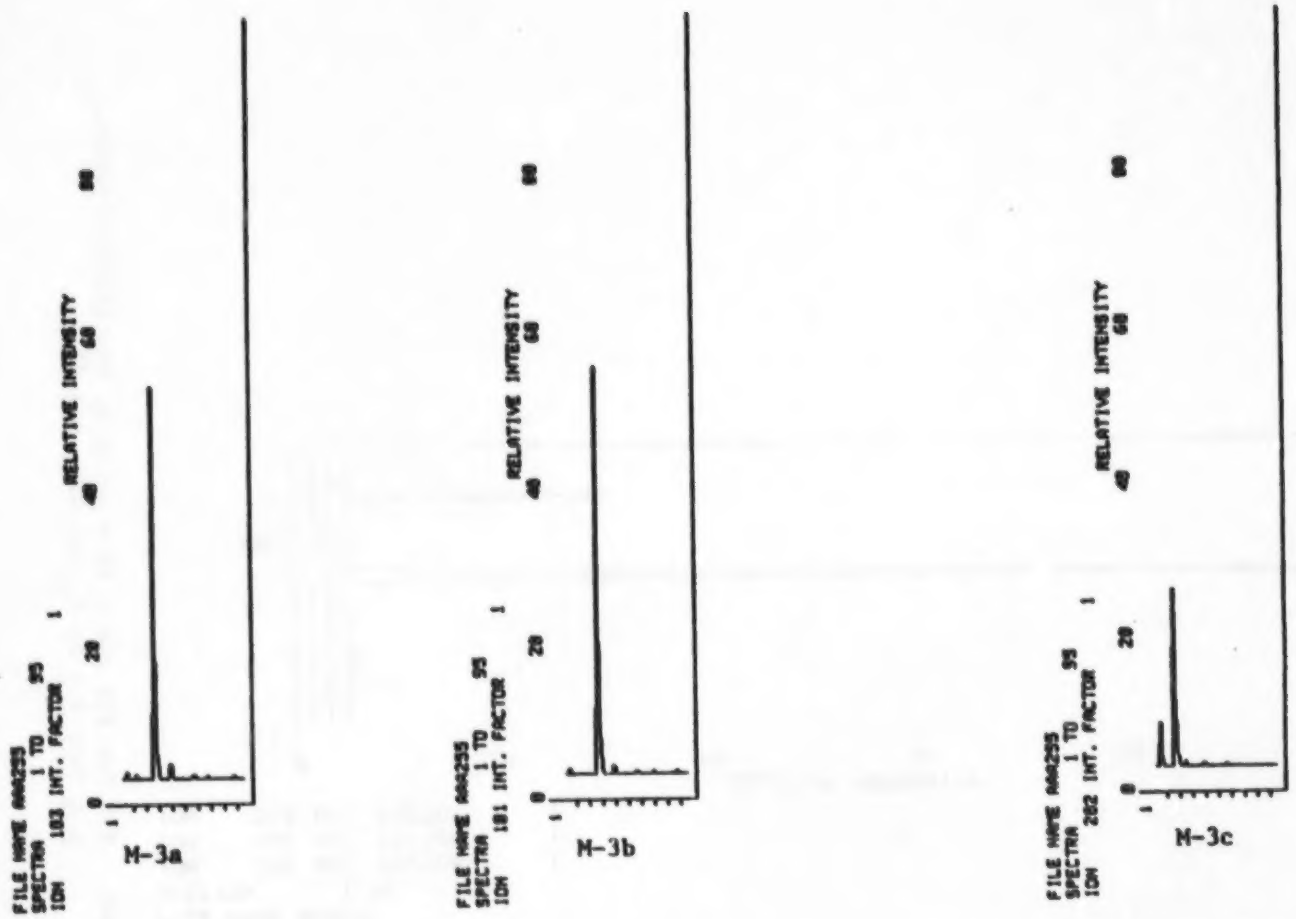


Figure M-3a. SIP for m/z 183 of lindane 100 ng/µl; M-3b. SIP for m/z 181 of lindane 100 ng/µl; M-3c. sip for m/z 202 of atrazine 100 ng/µl.

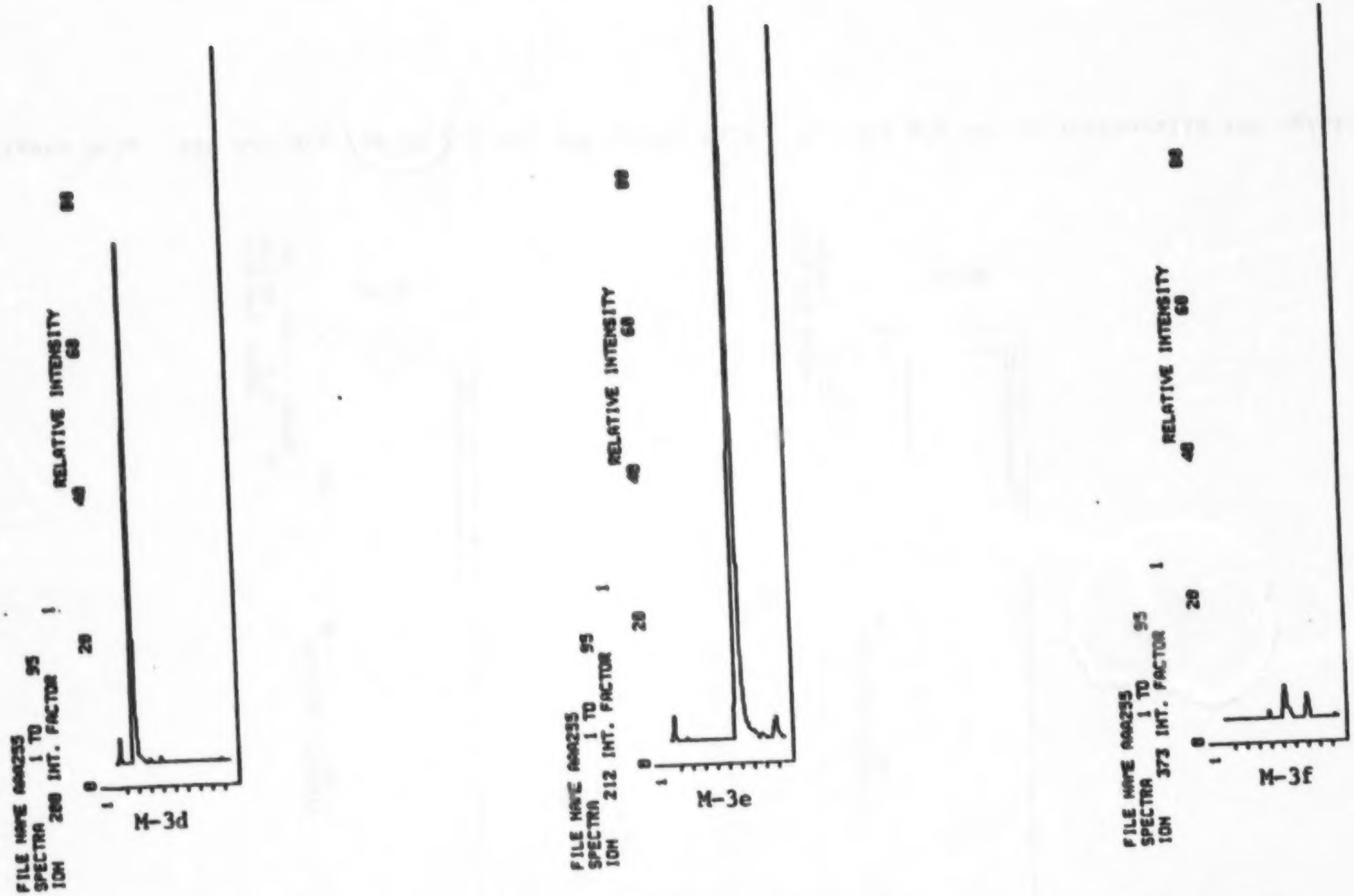


Figure M-3d. SIP for m/z 200 of atrazine 100 ng/µl; M-3e. SIP for m/z 212 of $d_{10}$-pyrene; M-3f. SIP for m/z 373 of chlordane 100 ng/µl.

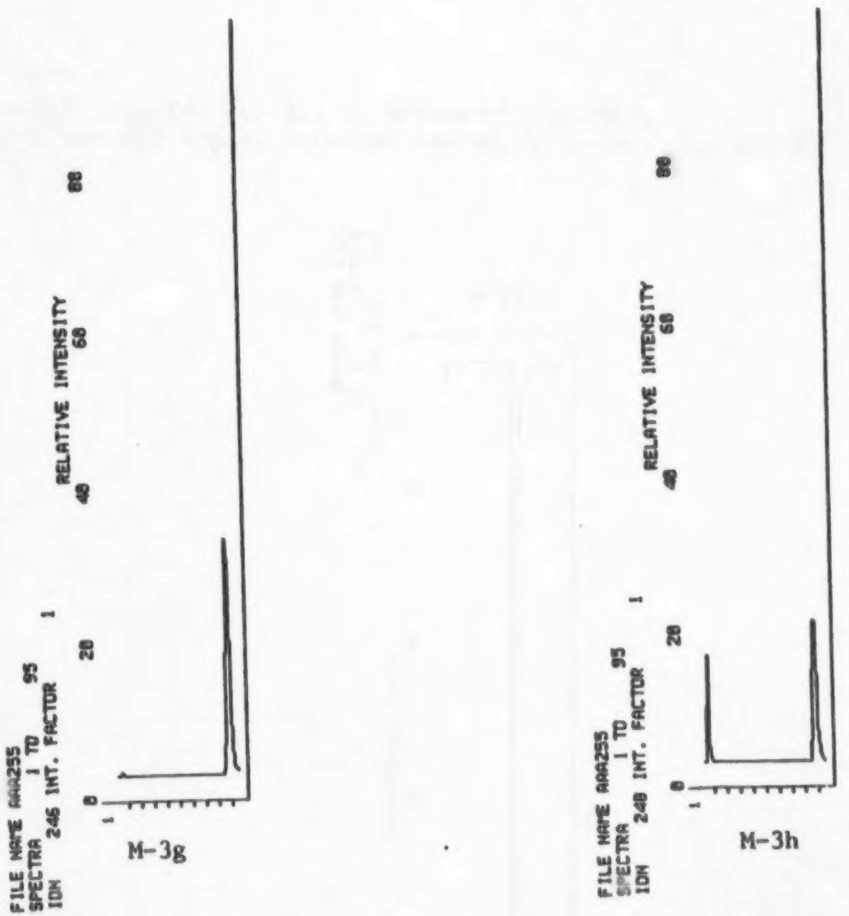


Figure M-3g. SIP for *m/z* 246 of *p*,*p*-DDE 100 ng/µl; M-3h. SIP for *m/z* 264 of trifluralin 100 ng/µl.

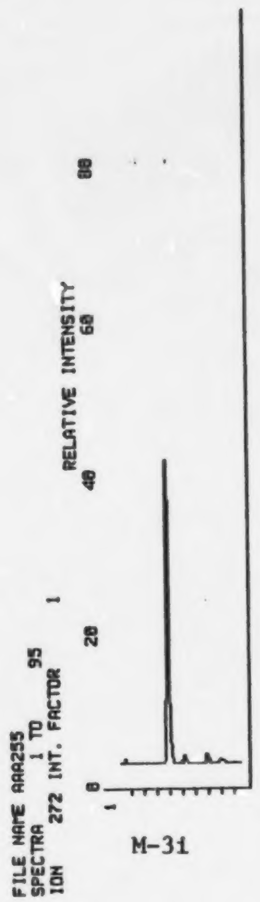


Figure M-31. SIP for m/z 272 of heptachlor 100 ng/μl.

# APPENDIX N

# DATA COLLECTION INSTRUMENTS UTILIZED IN PRETEST

RESEARCH TRIANGLE INSTITUTE
ASSESSMENT OF ORGANIC COMPOUNDS IN MAN AND ENVIRONMENTAL MEDIA

# PARTICIPANT CONSENT FORM

I understand that the Research Triangle Institute is engaged in a study of the exposure and absorption of selected organic compounds by persons living in areas having various levels of selected organic compounds in the environment. I understand that the survey is being conducted in order to help measure the levels of exposure and absorption of selected organic compounds in populations environmentally exposed, and is limited to the purpose stated. I further understand that the survey is being conducted under the auspices of the United States Environmental Protection Agency in cooperation with the New York State and Niagara County Health Departments.

I do hereby freely consent to participate in this study of exposure and absorption of selected organic compounds and understand that my participation will consist of providing answers to a questionnaire related to environmental exposure and the following environmental and biological samples: (1) three four ounce samples of cold tap water from a source commonly used for drinking and cooking, (2) a sample of environmental exposure collected by a small device which I will keep with me for a short time, (3) a small (approximately 25 cc) blood sample to be taken from an arm vein, (4) a breath sample, and (5) two small (approximately four ounce) urine samples, one to be taken early in the morning and one at the time of the blood sample. I understand that an agent of the Research Triangle Institute will administer the questionnaire in my home and at the same time collect the tap water samples, instruct me regarding the exposure monitoring device, and make arrangements regarding collection of the breath, blood, and urine samples.

I understand that my name will not be voluntarily disclosed, and that my name will not be referred to in any way when compiling and evaluating the results of the study. I understand that participation in this study may result in no direct benefits to me, and that I am free to withdraw from this study at any time. It has been explained to me that there are no significant risks to me from participation in this study. I further understand that while participating in the study I will be free to ask any questions concerning the study; if I have any further questions about the project, I know that I am free to contact Mr. Benjamin S. H. Harris, III, Survey Operations Center, Research Triangle Institute, Research Triangle Park, North Carolina 27709, telephone number 800-334-8571, extension 6055.

**Date:** ☐☐ – ☐☐ – ☐☐ *(Month)* *(Day)* *(Year)*     **Participant's Name:** ______________________ *(Print)*

**Site Number:** ☐     **Area Number:** ☐     **Participant Number:** ☐☐

**SIGNATURES:**

**Participant:** ______________________     **Witness:** ______________________

**Address:** ______________________     **Interviewer Number:** ☐☐☐

# ASSESSMENT OF HALOGENATED ORGANIC COMPOUNDS IN MAN AND ENVIRONMENTAL MEDIA

**Sponsored by:**

Office of Toxic Substances
Environmental Protection Agency
Washington, D.C. 20460

**Conducted by:**

Research Triangle Institute
P.O. Box 12194
Research Triangle Park, North Carolina 27709

# QUESTIONNAIRE

THE RESEARCH TRIANGLE INSTITUTE OF RESEARCH TRIANGLE PARK, NORTH CAROLINA, IS UNDERTAKING A RESEARCH STUDY FOR THE U.S. ENVIRONMENTAL PROTECTION AGENCY TO ASSESS LEVELS AND RELATIONSHIPS OF HALOGENATED ORGANIC COMPOUNDS IN MAN AND ENVIRONMENTAL MEDIA. THE INFORMATION RECORDED IN THIS QUESTIONNAIRE WILL BE HELD IN STRICT CONFIDENCE AND WILL BE USED SOLELY FOR RESEARCH INTO THE EFFECTS OF ENVIRONMENTAL FACTORS ON PUBLIC HEALTH. ALL RESULTS WILL BE SUMMARIZED FOR GROUPS OF PEOPLE; NO INFORMATION ABOUT INDIVIDUAL PERSONS WILL BE RELEASED WITHOUT THE CONSENT OF THE INDIVIDUAL. THIS QUESTIONNAIRE IS AUTHORIZED BY LAW (P.L. 94-469). WHILE YOU ARE NOT REQUIRED TO RESPOND, YOUR COOPERATION IS NEEDED TO MAKE THE RESULTS OF THIS SURVEY COMPREHENSIVE, ACCURATE, AND TIMELY.

**Study number:** ☐☐☐☐☐☐

**Site number:** ☐ **Area number:** ☐☐ **Participant number:** ☐☐

**First, I would like to ask some general questions about you.**

1. Sex *(by observation)*: ☐ 1 Male ☐ 2 Female

2. Race: ☐ 1 Hispanic ☐ 2 American Indian/ Alaskan Native ☐ 3 Black, not of Hispanic origin ☐ 4 Asian/Pacific Islander ☐ 5 White, not of Hispanic origin ☐ 6 Other *(Specify)* ____________

3. What was your age in years at last birthday? ☐☐ Years

4. What is your birthdate? *(Month)* ☐☐ - *(Day)* ☐☐ - *(Year)* ☐☐

5. What is your approximate weight in pounds? ☐☐☐ lbs. ☐ 1 Do not know

6. What is your approximate height in feet and inches? ☐ Feet ☐☐ Inches

---

**Next, I would like to ask some questions about your occupation.**

7. Are you presently employed in any capacity? ☐ 1 Yes *(Continue)* ☐ 2 No *(Go to Q. 11)*

8. How long have you been employed by your present employer? ☐☐ Units ☐ 1 Days ☐ 2 Months ☐ 3 Years

9. Does your occupation usually take you away from home? ☐ 1 Yes *(Continue)* ☐ 2 No *(Go to Q. 12)*

10. What is the nature and location (street address) of the company for which you work?

*(Specify)* ____________________________________ ☐☐☐☐☐ *(Zip Code)*

11. If not presently employed, which of the following best describes your status?

☐ 1 Housewife
☐ 2 Student
*(Go to Q. 15)*

☐ 3 Unemployed
☐ 4 Retired
☐ 5 Disabled
*(Continue)*

12. What is/was your usual occupation? *(Specify)* ____________________________

13. Are you presently employed in this occupation? ☐ 1 Yes ☐ 2 No

14. If yes to above question, how long have you been employed in that occupation?

*(Questions 13 and 14 may be skipped for unemployed, retired, and disabled persons.)* ☐☐ Units ☐ 1 Days ☐ 2 Months ☐ 3 Years

15. Do you work at or in any of the following occupations or establishments? *(Check all that apply.)*

☐ 1 Painting
☐ 2 Dry cleaning
☐ 3 Chemical plant
☐ 4 Petroleum plant
☐ 5 Service station/ garage/engine repair
☐ 6 Furniture refinishing or repair
☐ 7 None of these
☐ 8 Other *(Specify)* ____________

16. Have you worked at any of the following occupations/businesses at any time during the past week? *(Check all that apply.)*

☐ 1 Painting
☐ 2 Dry cleaning
☐ 3 Chemical plant
☐ 4 Petroleum plant
☐ 5 Service station/ garage/engine repair
☐ 6 Furniture refinishing or repair
☐ 7 None of these
☐ 8 Other *(Specify)* ____________

**Next, I would like to ask some questions regarding your personal habits.**

**17. Do you smoke?** ☐ 1 Yes *(Continue)* ☐ 2 No *(Go to Q. 20)*

**18. How old were you when you first started smoking?** ☐☐ Years

**19. On the average, how many cigarettes do you smoke per day?**

☐ 1 Less than ½ pack (1-4 cigarettes)
☐ 2 About ½ pack (5-14 cigarettes)
☐ 3 About 1 pack (15-24 cigarettes)
☐ 4 About 1½ packs (25-34 cigarettes)
☐ 5 About 2 packs (35-49 cigarettes)
☐ 6 More than 2 packs (50 or more cigarettes)

**NOTE:** ***If the participant uses tobacco in some other form (other than cigarettes—e.g., cigars, pipe, or snuff), record here:***

**20. What is the average number of hours that you spend out of doors each day?** ☐☐ Hours

**21. How many hours of the day, on the average, do you normally spend away from home?** ***(Average separately for weekdays and weekends.)***

Weekdays Hours ☐☐ Weekends Hours ☐☐

**22. Do you pump your own gas?** ☐ 1 Yes ☐ 2 No

***If yes, when did you last do so?*** *(Month)* ☐☐ - *(Day)* ☐☐ - *(Year)* ☐☐

**23. Do you do your own dry cleaning?** ☐ 1 Yes ☐ 2 No

***If yes, when did you last do so?*** *(Month)* ☐☐ - *(Day)* ☐☐ - *(Year)* ☐☐

**24. How many eggs have you eaten in the past 48 hours?** ☐☐.

**25. Do you pursue any of the following hobbies?** ***(Check all that apply.)***

☐ 1 Furniture refinishing ☐ 2 Painting ☐ 3 Scale models ☐ 4 Gardening ☐ 5 None of these

***If gardening is indicated, inquire about pesticide(s) used; if other hobbies are indicated, inquire about special solvents. (Specify)***

**26. Do you pursue any activity that includes regular use of solvent glue or model airplane cement?**

☐ 1 Yes ☐ 2 No

**Next, I would like to ask some questions regarding your health.**

**27. What do you consider the current status of your health?** ***(Check one.)***

☐ 1 **Excellent** ☐ 2 **Good** ☐ 3 **Fair** ☐ 4 **Poor**

**28. Are you currently taking any prescription medication(s) on a regular daily basis?** ☐ 1 **Yes** ☐ 2 **No**

***If yes, specify:*** ____________________

**29. Have you taken any non-prescription medications in the past 48 hours?** ☐ 1 **Yes** ☐ 2 **No**

***If yes, specify:*** ____________________

**30. Are you presently under a doctor's care?** ☐ 1 **Yes** ☐ 2 **No**

***If yes, specify reason:*** ____________________

**31. Are you presently suffering from any respiratory problems** ***(such as cold, cough, sore throat, flu, asthma, bronchitis, shortness of breath, laryngitis, pleurisy, etc.)?***

☐ 1 **Yes** ☐ 2 **No**

***If yes, specify:*** ____________________

**32. Have you ever been treated for anemia?** ☐ 1 **Yes** ☐ 2 **No**

---

**Lastly, I would like to ask some questions about your residence and household.**

**33. How many years have you lived in this area?** ☐☐ **Years**

**34. How long have you lived at your current address?** ☐☐ **Units** ☐ 1 **Days** ☐ 2 **Months** ☐ 3 **Years**

**35. Do you cool your home with any of the following appliances?** ***(Check all that apply.)***

☐ 1 **Central air conditioning**
☐ 2 **Window air conditioner(s)**
☐ 3 **Evaporative cooler(s)**
☐ 4 **Window fan(s)**
☐ 5 **Ceiling exhaust fan(s)**
☐ 6 **Circulating fan(s)**
☐ 7 **None of these**
☐ 8 **Do not know**
☐ 9 **Other** ***(Specify)*** ____________________

**36. Does your household grow any of its own food in a home garden?** ☐ 1 **Yes** ☐ 2 **No** ☐ 3 **Do not know**

***If yes, specify location of garden*** ____________________

**37. Where does your household obtain fresh fruit and/or vegetables?** ***(Specify)*** ____________________

**38. What is the primary source of your water for drinking?**

☐ 1 **Bottled water**
☐ 2 **Tap - municipal supply**
☐ 3 **Tap - community well**
☐ 4 **Tap - private well**
☐ 5 **Tap - cistern**
☐ 6 **Do not know**
☐ 7 **Other** ***(Specify)*** ____________________

**39. Is that the same primary source of water for drink mixes such as coffee, tea, Kool-Aid, etc?**

☐ 1 **Yes** ☐ 2 **No** ***If no, how does it differ? (Specify)*** ____________________

40. What is the primary source of your water for cooking?

☐ 1 Bottled water
☐ 2 Tap - municipal supply
☐ 3 Tap - community well
☐ 4 Tap - private well
☐ 5 Tap - cistern
☐ 6 Do not know
☐ 7 Other (Specify) ______________________

41. Does anyone else in your household smoke? ☐ 1 Yes ☐ 2 No ☐ 3 Do not know

*If yes, check all that apply:* ☐ 1 Cigarettes ☐ 2 Cigars ☐ 3 Pipe ☐ 4 Other (Specify) ______________________

42. Does anyone else in your household work at any of the following occupations/businesses? *(Check all that apply.)*

☐ 1 Painting
☐ 2 Dry cleaning
☐ 3 Chemical plant
☐ 4 Petroleum plant
☐ 5 Service station/garage/engine repair
☐ 6 Furniture refinishing or repair
☐ 7 None of these
☐ 8 Other (Specify) ______________________

43. Does anyone else in your household pursue any of the following hobbies? *(Check all that apply.)*

☐ 1 Painting ☐ 2 Furniture refinishing ☐ 3 Scale models ☐ 4 Gardening ☐ 5 None of these

---

## INTERVIEWER INFORMATION

44. Interviewer number: ☐☐☐

45. Date of interview: (Month) ☐☐ - (Day) ☐☐ - (Year) ☐☐

## COMMENTS

## SAMPLE INFORMATION

46. Personal monitor number ☐

| | Date | | | Time | Flow Rate (ml/min) | Interviewer Number |
|---|---|---|---|---|---|---|
| | Month | Day | Year | Hours : Minutes | | |
| On | | | | : | | |
| Off | | | | : | | |

COMMENTS:

47. Breath sample

| Collected | | Data on Collected Sample | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Date | | | Time | | Temp. C F | Volume Meter Reading (cubic feet) | Interviewer Number |
| | | | | | Start | Stop | | | |
| Yes | No | Month | Day | Year | Hours : Minutes | Hours : Minutes | | | |
| 1 | 2 | | | | : | : | | | |
| | If not collected, reason: | | | | | | | | |

Apparatus number ☐

48. Blood sample(s)

| Sample No. 1 | | Sample No. 2 | | Sample No. 3 | | All Sample(s) | | | | Interviewer Number |
|---|---|---|---|---|---|---|---|---|---|---|
| Collected | | Collected | | Collected | | If Collected, Date | | | If Not Collected, Reason | |
| Yes | No | Yes | No | Yes | No | Month | Day | Year | | |
| 1 | 2 | 1 | 2 | 1 | 2 | | | | | |

49. Urine sample(s)

| Early Morning Sample | | | | | | Interviewer Number | Spot Sample | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collected | | If Collected, Date | | | If Not Collected, Reason | | Collected | | If Collected, Date | | | If Not Collected, Reason |
| Yes | No | Month | Day | Year | | | Yes | No | Month | Day | Year | |
| 1 | 2 | | | | | | 1 | 2 | | | | |

50. Specific gravity: ☐.☐☐☐

51. Specific gravity: ☐.☐☐☐

52. Tap water sample(s) SOURCE: ______

| Sample No. 1 | | Sample No. 2 | | Sample No. 3 | | All Sample(s) | | | | Interviewer Number |
|---|---|---|---|---|---|---|---|---|---|---|
| Collected | | Collected | | Collected | | If Collected, Date | | | If Not Collected, Reason | |
| Yes | No | Yes | No | Yes | No | Month | Day | Year | | |
| 1 | 2 | 1 | 2 | 1 | 2 | | | | | |

### COMMENTS

# APPENDIX O

# DATA COLLECTION INSTRUMENTS PROPOSED FOR MAIN STUDY

## RESEARCH TRIANGLE INSTITUTE

## ASSESSMENT OF HALOGENATED ORGANIC COMPOUNDS IN MAN AND ENVIRONMENTAL MEDIA

## PARTICIPANT CONSENT FORM

I understand that the Research Triangle Institute is engaged in a study of the exposure and absorption of selected halogenated organic compounds by persons living in areas having various levels of selected halogenated organic compounds in the environment. I understand that the survey is being conducted in order to help measure the levels of exposure and absorption of selected halogenated organic compounds in populations environmentally exposed, and is limited to the purpose stated. I further understand that the survey is being conducted under the auspices of the United States Environmental Protection Agency in cooperation with the

*(State and local health departments).* ______________________________

I do hereby freely consent to participate in this study of exposure and absorption of selected halogenated organic compounds and understand that my participation will consist of providing answers to a questionnaire related to environmental exposure and the following environmental and biological samples: (1) two four ounce samples of cold tap water from a source commonly used for drinking and cooking, (2) a small (approximately 25 cc) blood sample to be taken from an arm vein, (3) a breath sample, and (4) a small (approximately four ounce) urine sample to be taken early in the morning. I understand that an agent of the Research Triangle Institute will administer the questionnaire in my home and at the same time collect the tap water samples and make arrangements regarding collection of the breath, blood, and urine samples. I understand that after the collection of the breath and blood samples I will receive an incentive of ten dollars for my full participation in the study. I further understand that a small sample of participants will be requested to provide an additional 25 cc blood sample at a different time and that, if I am so selected and agree to provide this additional sample, I will receive an additional incentive of five dollars for my assistance. I understand that a small number of households and individuals will be selected for reinterview and the collection of duplicate tap water and blood (10 cc additional to be collected at the same time as the original sample) samples, but that such selection would not entitle me to further compensation.

I understand that my name will not be voluntarily disclosed, and that my name will not be referred to in any way when compiling and evaluating the results of the study. I understand that participation in this study may result in no direct benefits to me, other than those described herein, and that I am free to withdrew from this study at any time. It has been explained to me that there are no significant risks to me from participation in this study. I further understand that while participating in the study I will be free to ask any questions concerning the study; if I have any further questions about the project, I know that I am free to contact

*(Local health department representative)* ______________________ telephone number ______________________ or

*(State health department representative)* ______________________ telephone number ______________________

or Mr. Benjamin S. H. Harris, III, Survey Operations Center, Research Triangle Institute, Research Triangle Park, North Carolina 27709, telephone number 800-334-8571, extension 6065.

**Date:** ☐☐ - ☐☐ - ☐☐ *(Month) (Day) (Year)* **Participant's Name:** ______________________ *(Print)*

**Area Number:** ☐ **Site Number:** ☐ **Segment Number:** ☐☐ **Household Number:** ☐☐☐

**SIGNATURES:**

**Participant:** ______________________ **Witness:** ______________________

**Participant Number:** ☐☐ **Interviewer Number:** ☐☐☐

# ASSESSMENT OF HALOGENATED ORGANIC COMPOUNDS IN MAN AND ENVIRONMENTAL MEDIA

**Sponsored by:**

Office of Toxic Substances
Environmental Protection Agency
Washington, D.C. 20460

**Conducted by:**

Research Triangle Institute
P.O. Box 12194
Research Triangle Park, North Carolina 27709

# QUESTIONNAIRE

THE RESEARCH TRIANGLE INSTITUTE OF RESEARCH TRIANGLE PARK, NORTH CAROLINA, IS UNDERTAKING A RESEARCH STUDY FOR THE U.S. ENVIRONMENTAL PROTECTION AGENCY TO ASSESS LEVELS AND RELATIONSHIPS OF HALOGENATED ORGANIC COMPOUNDS IN MAN AND ENVIRONMENTAL MEDIA. THE INFORMATION RECORDED IN THIS QUESTIONNAIRE WILL BE HELD IN STRICT CONFIDENCE AND WILL BE USED SOLELY FOR RESEARCH INTO THE EFFECTS OF ENVIRONMENTAL FACTORS ON PUBLIC HEALTH. ALL RESULTS WILL BE SUMMARIZED FOR GROUPS OF PEOPLE; NO INFORMATION ABOUT INDIVIDUAL PERSONS WILL BE RELEASED WITHOUT THE CONSENT OF THE INDIVIDUAL. THIS QUESTIONNAIRE IS AUTHORIZED BY LAW (P.L. 94-469). WHILE YOU ARE NOT REQUIRED TO RESPOND, YOUR COOPERATION IS NEEDED TO MAKE THE RESULTS OF THIS SURVEY COMPREHENSIVE, ACCURATE, AND TIMELY.

**Study number:** ☐☐☐☐☐☐

**Area number:** ☐ **Site number:** ☐ **Segment number:** ☐☐ **Household number:** ☐☐☐

**Participant number:** ☐☐

**First, I would like to ask some general questions about you.**

1. Sex *(By observation):* ☐ 1 Male ☐ 2 Female

2. Race: ☐ 1 Hispanic ☐ 2 American Indian/Alaskan Native
☐ 3 Black, not of Hispanic origin ☐ 4 Asian/Pacific Islander
☐ 5 White, not of Hispanic origin ☐ 6 Other *(Specify)* __________

3. What was your age in years at last birthday? ☐☐ Years

4. What is your birthdate?
*(Month)* ☐☐ - *(Day)* ☐☐ - *(Year)* ☐☐

5. What is your approximate weight in pounds?
☐☐☐ lbs. ☐ 1 Do not know

6. What is your approximate height in feet and inches?
☐ Feet ☐☐ Inches

---

**Next, I would like to ask some questions about your occupation.**

7. Are you presently employed in any capacity? ☐ 1 Yes *(Continue)* ☐ 2 No *(Go to Q. 11)*

8. How long have you been employed by your present employer? ☐☐ Units ☐ 1 Days ☐ 2 Months ☐ 3 Years

9. Does your occupation usually take you away from home? ☐ 1 Yes *(Continue)* ☐ 2 No *(Go to Q. 12)*

10. What is the nature and location (street address) of the company for which you work?
*(Specify)* ____________________ ☐☐☐☐☐ *(Zip Code)* *(Go to Q. 12)*

11. If not presently employed, which of the following best describes your status?
☐ 1 Housewife
☐ 2 Student
} *(Go to Q. 15)*
☐ 3 Unemployed
☐ 4 Retired
☐ 5 Disabled
} *(Continue)*

12. What is/was your usual occupation? *(Specify)* ____________________

*(Questions 13 and 14 may be skipped for unemployed, retired, and disabled persons.)*

13. Are you presently employed in this occupation? ☐ 1 Yes *(Continue)* ☐ 2 No *(Go to Q. 15)*

14. If yes to above question, how long have you been employed in that occupation?
☐☐ Units ☐ 1 Days ☐ 2 Months ☐ 3 Years

15. Have you ever worked at or in any of the following occupations or establishments? *(Check all that apply.)*
☐ 1 Painting ☐ 3 Chemical plant ☐ 5 Service station/garage/engine repair ☐ 7 Plastics manufacture or formulation ☐ 9 Wood processing plant
☐ 2 Dry cleaning ☐ 4 Petroleum plant ☐ 6 Furniture refinishing or repair ☐ 8 Textile mill ☐ 0 None of these *(Go to Q. 17)*

16. Have you wo ked at any of the following occupations/businesses at any time during the past week? *(Check all that apply.)*
☐ 1 Painting ☐ 3 Chemical plant ☐ 5 Service station/garage/engine repair ☐ 7 Plastics manufacture or formulation ☐ 9 Wood processing plant
☐ 2 Dry cleaning ☐ 4 Petroleum plant ☐ 6 Furniture refinishing or repair ☐ 8 Textile mill ☐ 0 None of these

**Next, I would like to ask some questions regarding your personal habits.**

17. Have you ever smoked as many as 5 packs of cigarettes—that is, as many as 100 cigarettes during your entire life?

☐1 Yes *(Continue)* ☐2 No *(Go to Q. 22)*

18. Do you now smoke cigarettes? ☐1 Yes *(Continue)* ☐2 No *(Go to Q. 20)*

19. How old were you when you first started smoking? ☐☐ Years *(Go to Q. 21)*

20. If you no longer smoke, how old were you when you last gave up smoking? ☐☐ Years

21. On the average, how many cigarettes do (did) you smoke per day?

☐1 Less than ½ pack (1-4 cigarettes)
☐2 About ½ pack (5-14 cigarettes)
☐3 About 1 pack (15-24 cigarettes)
☐4 About 1½ packs (25-34 cigarettes)
☐5 About 2 packs (35-49 cigarettes)
☐6 More than 2 packs (50 or more cigarettes)

22. Do you use tobacco in any other form *(e.g., cigars, pipe, snuff, chewing tobacco)*? ☐1 Yes ☐2 No

*If yes, indicate form:* ☐1 Cigars ☐2 Snuff ☐3 Chewing tobacco

☐4 Pipe ☐5 Other *(Specify)* ______________________________

23. What is the average number of hours that you spend out of doors each day? ☐☐ Hours

24. How many hours of the day, on the average, do you normally spend away from home? *(Average separately for weekdays and weekends.)*

| | |
|---|---|
| Weekdays | Hours ☐☐ |
| Weekends | Hours ☐☐ |

25. Do you pump your own gas? ☐1 Yes ☐2 No

*If yes, have you pumped your own gas in the last 24 hours?* ☐1 Yes ☐2 No

26. Do you do your own dry cleaning? ☐1 Yes ☐2 No

27. Have you been to a dry cleaning establishment in the past 24 hours? ☐1 Yes ☐2 No

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best available copy.

28. Do you pursue any of the following hobbies? *(Check all that apply.)*

☐ 1 Furniture refinishing ☐ 2 Painting ☐ 3 Scale models ☐ 4 Gardening ☐ 5 None of these

29. Do you work with or use insecticides, pesticides, or herbicides, as in farming, gardening, or extermination?

☐ 1 Yes ☐ 2 No

*If yes,* a. How often would you say that you work with or use such substances? ☐ 1 Rarely ☐ 2 Occasionally ☐ 3 Often

b. When did you last work with such substances? *(Month)* ☐☐ - *(Day)* ☐☐ - *(Year)* ☐☐

---

**Next, I would like to ask some questions regarding your health.**

30. What do you consider the current status of your health? *(Check one.)*

☐ 1 Excellent ☐ 2 Good ☐ 3 Fair ☐ 4 Poor

31. Are you currently taking any prescription medication(s) on a regular daily basis? ☐ 1 Yes ☐ 2 No

*If yes, specify:* ______________________________

32. Have you taken any non-prescription medications in the past 48 hours? ☐ 1 Yes ☐ 2 No

*If yes, specify:* ______________________________

33. Are you presently under a doctor's care? ☐ 1 Yes ☐ 2 No

*If yes, specify reason(s):* ______________________________

34. Are you presently suffering from any respiratory problems *(such as cold, cough, sore throat, flu, asthma, bronchitis, shortness of breath, laryngitis, pleurisy, etc.)?*

☐ 1 Yes ☐ 2 No

*If yes, specify:* ______________________________

35. Have you ever been treated for any of the following conditions?

☐ 1 Anemia ☐ 2 Liver disease ☐ 3 Kidney disease

36. How would you rate your general recreational activity?

☐ 1 Heavy ☐ 2 Light ☐ 3 Sedentary

37. How would you rate your activity on the job?

☐ 1 Heavy ☐ 2 Light ☐ 3 Sedentary ☐ 4 Not applicable *(Retired, unemployed, or disabled)*

**Next, I would like to ask some questions regarding your diet.**

38. Which meal(s) do you usually eat at home? *(Check all that apply.)*

☐ 1 Breakfast ☐ 2 Lunch ☐ 3 Dinner ☐ 4 None

39. When you do not eat at home, where do you eat? *(Check all that apply.)*

☐ 1 Meal prepared at home but eaten elsewhere ☐ 2 School ☐ 3 Work ☐ 4 Restaurant

☐ 5 Never eat anywhere but at home ☐ 6 Other *(Specify)* ____________________

40. Are you presently following any of the following dietary regimens? *(Check all that apply.)*

☐ 1 Bland food ulcer diet ☐ 4 Organic foods ☐ 7 Other *(Specify)* ____________________

☐ 2 Diabetic diet ☐ 5 Vegetarian ____________________

☐ 3 Reducing diet ☐ 6 None of these ____________________

____________________

41. On the average, how often do you eat the following foods? *(Check each appropriate box.)*

| Foodstuff | Daily 1 | More than 3 times per week 2 | About once per week 3 | Once every 2 weeks 4 | Once per month 5 | Less than once per month 6 |
|---|---|---|---|---|---|---|
| a. Beef | | | | | | |
| b. Fish | | | | | | |
| c. Pork | | | | | | |
| d. Poultry | | | | | | |
| e. Fresh fruit | | | | | | |
| f. Frozen fruit | | | | | | |
| g. Canned fruit | | | | | | |
| h. Fresh vegetables | | | | | | |
| i. Frozen vegetables | | | | | | |
| j. Canned vegetables | | | | | | |

---

**Lastly, I would like to ask some questions about your residence and household.**

42. How many years have you lived in this area? ☐☐ Years

43. How long have you lived at your current address? ☐☐ Units ☐ 1 Days ☐ 2 Months ☐ 3 Years

44. Do you cool your home with any of the following appliances? *(Check all that apply.)*

☐ 1 Central air conditioning ☐ 4 Window fan(s) ☐ 7 None of these

☐ 2 Window air conditioner(s) ☐ 5 Ceiling exhaust fan(s) ☐ 8 Do not know

☐ 3 Evaporative cooler(s) ☐ 6 Circulating fan(s) ☐ 9 Other *(Specify)* ____________________

45. Does your household grow any of its own food in a home garden? ☐ 1 Yes ☐ 2 No ☐ 3 Do not know

*If yes, specify location of garden:* ______________________________

46. Where does your household obtain fresh fruit and/or vegetables? *(Specify)* ______________________________

47. What is the primary source of your water for drinking?

- ☐ 1 Bottled water
- ☐ 2 Tap - municipal supply
- ☐ 3 Tap - community well
- ☐ 4 Tap - private well
- ☐ 5 Tap - cistern
- ☐ 6 Do not know
- ☐ 7 Other *(Specify)* ______________________________

48. Is that the same primary source of water for drink mixes such as coffee, tea, Kool-Aid, etc.?

☐ 1 Yes ☐ 2 No *If no, how does it differ? (Specify)* ______________________________

49. What is the primary source of your water for cooking?

- ☐ 1 Bottled water
- ☐ 2 Tap - municipal supply
- ☐ 3 Tap - community well
- ☐ 4 Tap - private well
- ☐ 5 Tap - cistern
- ☐ 6 Do not know
- ☐ 7 Other *(Specify)* ______________________________

50. Does anyone (else) in your household smoke? ☐ 1 Yes ☐ 2 No ☐ 3 Do not know

*If yes, check all that apply:* ☐ 1 Cigarettes ☐ 2 Cigars ☐ 3 Pipe ☐ 4 Other *(Specify)* ______________________________

51. Does anyone (else) in your household work at any of the following occupations/businesses? *(Check all that apply.)*

- ☐ 1 Painting
- ☐ 2 Dry cleaning
- ☐ 3 Chemical plant
- ☐ 4 Petroleum plant
- ☐ 5 Service station/garage/engine repair
- ☐ 6 Furniture refinishing or repair
- ☐ 7 Plastics manufacture or formulation
- ☐ 8 Textile mill
- ☐ 9 Wood processing plant
- ☐ 0 None of these

52. Does anyone (else) in your household pursue any of the following hobbies? *(Check all that apply.)*

☐ 1 Painting ☐ 2 Furniture refinishing ☐ 3 Scale models ☐ 4 Gardening ☐ 5 None of these

---

## INTERVIEWER INFORMATION

53. Interviewer number: ☐☐☐

54. Date of interview: ☐☐ - ☐☐ - ☐☐ *(Month)* *(Day)* *(Year)*

## COMMENTS

______________________________

______________________________

______________________________

______________________________

______________________________

______________________________

______________________________

______________________________

______________________________

## SAMPLE INFORMATION

*FOR EACH SAMPLE COLLECTED FOR A GIVEN HOUSEHOLD OR INDIVIDUAL, ATTACH THE APPROPRIATE LABEL TO THE APPROPRIATE CONTAINER BEFORE COLLECTING THE SAMPLE.*

### A. ORIGINAL SAMPLE COLLECTION

1. Outside air (household) monitor number ☐

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Data on Collected Sample | | | | | |
| | | | Date | | | Time | Flow Rate (ml/min) | Interviewer Number |
| Collected | | | Month | Day | Year | Hours : Minutes | | |
| Yes | No | On | | | | : | | |
| 1 | 2 | Off | | | | : | | |
| | If not collected, reason: | | | | | | | |

COMMENTS: ____________________

2. Soil sample

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Original Sample(s) | | | | | | Interviewer Number | Duplicate Sample | | | | |
| Collected | | If Collected, Date | | | If Not Collected, Reason | | Selected | | Collected | | If Selected, But Not Collected, Reason |
| Yes | No | Month | Day | Year | | | Yes | No | Yes | No | |
| 1 | 2 | | | | | | 1 | 2 | 1 | 2 | |

3. Tap water sample

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Original Sample(s) | | | | | | Interviewer Number | Duplicate Sample | | | | |
| Collected | | If Collected, Date | | | If Not Collected, Reason | | Selected | | Collected | | If Selected, But Not Collected, Reason |
| Yes | No | Month | Day | Year | | | Yes | No | Yes | No | |
| 1 | 2 | | | | | | 1 | 2 | 1 | 2 | |

Source: ____________________

Appointment date: ☐☐ - ☐☐ - ☐☐ (Month) (Day) (Year)

And time: ☐☐ : ☐☐ a.m. p.m.

4. Breath sample

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Data on Collected Sample | | | | | | | |
| | | Date | | | Time | | Temp. C F | Volume Meter Reading (cubic feet) | Interviewer Number |
| Collected | | | | | Start | Stop | | | |
| Yes | No | Month | Day | Year | Hours : Minutes | Hours : Minutes | | | |
| 1 | 2 | | | | : | : | | | |
| | If not collected, reason: | | | | | | | | |

Apparatus number ☐

5. Blood sample

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Original Sample | | | | | | Interviewer Number | Duplicate Sample | | | | |
| Collected | | If Collected, Date | | | If Not Collected, Reason | | Selected | | Collected | | If Selected, But Not Collected, Reason |
| Yes | No | Month | Day | Year | | | Yes | No | Yes | No | |
| 1 | 2 | | | | | | 1 | 2 | 1 | 2 | |

6. Urine sample(s)

| Early Morning Sample | | | | | | Interviewer Number | Other Sample *(Explain below)* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collected | | If Collected, Date | | | If Not Collected, Reason | | Collected | | If Collected, Date | | | If Not Collected, Reason |
| Yes | No | Month | Day | Year | | | Yes | No | Month | Day | Year | |
| 1 | 2 | | | | | | 1 | 2 | | | | |

7. Specific gravity: ☐.☐☐☐

8. Specific gravity: ☐.☐☐☐

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**B. ADDITIONAL SAMPLE**

Appointment date: *(Month)* ☐☐ – *(Day)* ☐☐ – *(Year)* ☐☐

And time: ☐☐ : ☐☐ a.m. p.m.

1. Have you pumped your own gas in the last 24 hours? ☐1 Yes ☐2 No
2. Have you been to a dry cleaning establishment in the last 24 hours? ☐1 Yes ☐2 No
3. Have you worked with or used any insecticides, pesticides, or herbicides in the past 24 hours? ☐1 Yes ☐2 No
4. Have you taken any non-prescription medications in the past 24 hours? ☐1 Yes ☐2 No

*If yes, specify:* ______________________________

5. Blood sample

| Original Sample | | | | | | Interviewer Number | Duplicate Sample | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Collected | | If Collected, Date | | | If Not Collected, Reason | | Selected | | Collected | | If Selected, But Not Collected, Reason |
| Yes | No | Month | Day | Year | | | Yes | No | Yes | No | |
| 1 | 2 | | | | | | 1 | 2 | 1 | 2 | |

**COMMENTS**

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